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Expedition Report: EX-21-02 2021 Technology Demonstration (AUV and Mapping)

U.S. Southeast
Cape Canaveral, Florida to Norfolk, Virginia
May 14 - 27, 2021

Authors:
Michael P. White[1]
Derek Sowers[1]
Katharine Egan[2]
Rachel Gulbraa[3]
Christa Rabenold[3]
Kim Galvez[1]

Other Contributors:
Timothy Shank[4]
Casey Machado[4]

[1] Cherokee Federal under contract to NOAA Ocean Exploration
[2] NOAA Ocean Exploration
[3] University Corporation for Atmospheric Research under contract to NOAA Ocean Exploration
[4] Woods Hole Oceanographic Institution (WHOI)

February 25, 2022
NOAA Ocean Exploration
1315 East-West Hwy, SSMC3 RM 10210
Silver Spring, MD 20910

Abstract

From May 14-27, 2021, NOAA Ocean Exploration led the 2021 Technology Demonstration on NOAA Ship *Okeanos Explorer* from Cape Canaveral, Florida, to Norfolk, Virginia. The expedition provided an opportunity to test several technologies that will allow the ocean exploration community to systematically explore hadal depths. The expedition brought together NOAA's Ocean Exploration Cooperative Institute (OECI), NOAA's Northwest Fisheries Science Center (NWFSC), NASA's Jet Propulsion Laboratory (NASA JPL), and two OECI member institutions — Woods Hole Oceanographic Institution (WHOI), and the Inner Space Center/University of Rhode Island (ISC/URO) —to advance new ocean technologies and sampling techniques. The expedition had three overall objectives: field testing and development of WHOI/NASA JPL Orpheus class autonomous underwater vehicles (AUVs), piloting environmental DNA (eDNA) collection for NOAA Ocean Exploration, and mapping priority deepwater areas offshore the U.S. Southeast, largely focused on the Blake Plateau. The Orpheus class AUV project was the first OECI-supported project to take place on a NOAA ship. During 14 days at sea, eight AUV deployments were completed between 12-866 meters depth. The AUVs surveyed 30 linear kilometers of seafloor, logged over 16 hours of bottom time, and collected over 724 GB of down-looking 4K video. The AUVs spent a total of 32 hours and 59 minutes in the water, which included water column exploration during descents and ascents. Twelve conductivity, temperature, and depth system (CTD) rosette casts were completed, most simultaneous with AUV operations. Using the Niskin bottles on the CTD rosette, 120 water samples and 13 control samples were collected and processed for post-expedition eDNA analysis. A detailed standard operating procedures document for eDNA collection was developed during the expedition. Exploration mapping operations included acoustic data collection using the EM 304 MKII multibeam echosounder, Simrad EK60 and EK80 split-beam sonar system, Knudsen Chirp 3260 sub-bottom profiler, and the acoustic Doppler current profiler (ADCP). 9,669 square kilometers of largely unmapped seafloor were mapped using the EM 304 within the U.S. Exclusive Economic Zone. Most of this bathymetric data was collected in water deeper than 200 meters. EX-21-02 took full advantage of high-bandwidth communications capability by enabling video streaming of AUV testing to onshore researchers and engineers. In addition, telepresence-enabled live interactions were held connecting onboard scientists to onshore audiences during live interview sessions.

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For further information, direct inquiries to:

NOAA Office of Ocean Exploration and Research
1315 East-West Hwy, SSMC3 RM 10210
Silver Spring, MD 20910
Phone: (301) 734-1014
Email: ex.expeditioncoordinator@noaa.gov

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1. Introduction

The NOAA Office of Ocean Exploration and Research (NOAA Ocean Exploration) is the only federal program dedicated to exploring the deep ocean, closing prominent gaps in our basic understanding of U.S. deep waters and the seafloor, and delivering the ocean information needed to strengthen the economy, health, and security of our nation.

Using the latest tools and technology, NOAA Ocean Exploration explores previously unknown areas of our deep ocean, making discoveries of scientific, economic, and cultural value. Through live video streams, online coverage, training opportunities, and real-time events, NOAA Ocean Exploration allows scientists, resource managers, students, members of the general public, and others to actively experience ocean exploration, expanding available expertise, cultivating the next generation of ocean explorers, and engaging the public in exploration activities. To better understand our ocean, NOAA Ocean Exploration makes exploration data available to the public. This allows us, collectively, to more effectively maintain ocean health, sustainably manage our marine resources, accelerate our national economy, and build a better appreciation of the value and importance of the ocean in our everyday lives.

NOAA Ocean Exploration regularly conducts technology demonstration expeditions like EX-21-02 as an opportunity to test new and emerging technologies and applications that can enhance the program's operations as well as advance the capabilities of the ocean exploration community at large. EX-21-02 focused on the development and testing of a new class of autonomous underwater vehicles (AUVs) capable of reaching hadal depths, piloting environmental DNA (eDNA) collection and processing, and mapping priority deepwater areas offshore the U.S. Southeast, largely focused on the Blake Plateau.

1.1 Atlantic Seafloor Partnership for Integrated Research and Exploration

Data collected during expeditions on NOAA Ship *Okeanos Explorer* from 2018-2021 directly contribute to the Atlantic Seafloor Partnership for Integrated Research and Exploration (ASPIRE), a major multiyear, multinational collaborative field program focused on raising collective knowledge and understanding of the North Atlantic Ocean. ASPIRE builds on the momentum of past U.S. campaigns and international initiatives to support ecosystem-based management of marine resources. ASPIRE also provides information relevant to NOAA's emerging Blue Economy priorities, which, in addition to ocean exploration, are seafood production, tourism and recreation, marine transportation, and coastal resilience.

2. Expedition Overview

From May 14, 2021 to May 27, 2021 NOAA Ocean Exploration and partners conducted a telepresence-enabled ocean exploration expedition on *Okeanos Explorer* to test emerging technologies, conduct a pilot sampling project, and collect critical baseline information and improve knowledge about unexplored and poorly understood deepwater areas of the U.S. Southeast. EX-21-02 was part of a series of expeditions contributing to ASPIRE. Previous expeditions in this region include EX-21-01 EM304 Sea Acceptance Testing and Mapping Shakedown[1], EX-19-07 and EX-19-06 2019 Southeastern U.S. Deep-sea Exploration[2], EX-1903 Leg 1 and EX-19-03 Leg 2 Windows to the Deep 2019[3], and EX-18-05 and EX-18-06 Windows to the Deep 2018: Exploration of the Southeast U.S. Continental Margin[4]. EX-21-02 was designed to demonstrate emerging technologies and sampling techniques and provide timely high-resolution mapping data in a high-priority area of the EEZ as identified in the 2018 ASPIRE Workshop[5] (NOAA, 2018). Like other ASPIRE expeditions, it also served as an opportunity for NOAA to highlight the uniqueness and importance of deepwater environments.

2.1 Rationale for Exploration

The deepwater areas offshore Florida, Georgia, South Carolina, and North Carolina are some of the least explored areas along the U.S. East Coast. Though this region is home to millions of Americans and is experiencing some of the highest population growth rates in the United States (Conley et al., 2017), there are large gaps in our understanding of offshore habitats. The southeastern U.S. continental margin has some of the largest gaps in high-resolution ocean mapping data on the East Coast.

As part of the planning for this expedition, NOAA Ocean Exploration collaborated with the scientific and management community to assess the exploration needs and data gaps in unknown and poorly known areas of the southeastern U.S. continental margin. To define the operating area for this expedition, NOAA Ocean Exploration considered the 2018 Call

[1] https://oceanexplorer.noaa.gov/okeanos/explorations/ex2101/welcome.html

[2] https://oceanexplorer.noaa.gov/okeanos/explorations/ex1907/welcome.html

[3] https://oceanexplorer.noaa.gov/okeanos/explorations/ex1903/welcome.html

[4] https://oceanexplorer.noaa.gov/okeanos/explorations/ex1806/welcome.html

[5] ASPIRE Workshop Report

for Input, results from the 2018 ASPIRE Workshop (NOAA, 2018), and priorities from resource managers.

Data and information from this expedition will help improve our understanding of the deep-ocean habitats of the U.S. continental margin and the connections between communities throughout the Atlantic basin. It will also inform deep-sea management plans for habitat areas of particular concern (HAPCs), marine protected areas (MPAs), and national marine sanctuaries; support local scientists and managers seeking to understand and manage deep-sea resources; and stimulate subsequent exploration, research, and management activities.

EX-21-02 also contributed to the National Strategy for Mapping, Exploring, and Characterizing the United States Exclusive Economic Zone[6] by collecting high-resolution multibeam data in unmapped areas, and collecting seafloor imagery, and water samples for eDNA analysis to support seafloor and water column characterization.

2.2 Rationale for Technology Demonstration

The 2021 Technology Demonstration provided an opportunity to test and improve ocean exploration technologies. Expeditions like this one are vital for the advancement of ocean exploration technologies that will benefit partners and the broader field of ocean exploration alike in our collective mission to explore, map, and understand the vast ocean realm. The technologies of today and those that are newly emerging for tomorrow are allowing us to explore the ocean with increasing efficiency and flexibility while continuing to minimize impact and disruption. Expedition priorities included completing field engineering trials of Woods Hole Oceanographic Institution (WHOI)'s autonomous underwater vehicles *Orpheus* and *Eurydice* and piloting eDNA field sampling protocols. This was the first project support by the Ocean Exploration Cooperative Institute (OECI) to take place onboard a NOAA vessel.

2.3 Rationale for Orpheus Class AUVs

Autonomous underwater vehicles (AUVs), are programmable and self-powered and can operate with no real-time control by, or physical connection to, their human operator. AUVs can carry a variety of sonars, sensors, and cameras, and are used to map the ocean floor, record environmental information, explore geologic formations, document shipwrecks, and more. AUVs can stay underwater for extended periods of time, carrying out repetitive tasks or tasks that might require constant adjustment.

[6] National Strategy for Mapping, Exploring, and Characterizing the United States Exclusive Economic Zone

Orpheus[7] is both the new class of AUV capable of reaching the hadal zone (depths greater than 6,000 meters) and the name of the first vehicle of the class (**Figures 1 and 2**). The minimalist design of the Orpheus class vehicles make them less costly than their more traditional alternatives, and their modular payload makes them able to be reconfigured to match a variety of mission profiles. These relatively small vehicles are designed with more of-the-shelf components to minimize costs. *Orpheus* and *Eurydice*, the second AUV of the class, were built in 2018. They were designed by Woods Hole Oceanographic Institution (WHOI) in collaboration with NASA's Jet Propulsion Laboratory (JPL) to withstand the pressure of hadal ocean depths, and also as a potential analog for deep space ocean discovery. Orpheus class AUVs are designed to be deployable from any sized ship of opportunity. Future concepts of operations with the AUVs include a swarm approach, where multiple AUVs are deployed and work in concert to efficiently collect a variety of ocean data over a larger area in a given time than a single platform could.

There are notable parallels between ocean and space exploration as the extreme environments of Earth's deep ocean can mimic extreme conditions in space, making our own ocean a good place to test new technologies for space exploration. By examining parallels in ocean and space exploration, we can develop better tools and technologies to study the physical and biological processes of the Earth's ocean, and potentially, those on other ocean worlds as well.

During the 2021 Technology Demonstration expedition, the team equipped the vehicles with a 4K downward-looking science camera and autonomous in-situ electrochemical sensing via an ISEA-X instrument (Analytical Instrument Systems). Both *Orpheus* and *Eurydice* have the following specifications:

- 11,000 meter (36,000 foot) depth rating
- Weight: 250 kg (550 lbs) out of water
- Dimensions: 68''L x 40''W x 51''H
- Visual terrain-relative navigation
- Non-disruptive seafloor landing and sampling
- Modular science payload bay that can include chemical and biological sensors and samples

[7] Additional background information: *Orpheus*, The Rise of *Orpheus*, HADEX Technology.

Figure 1. *Orpheus* AUV being launched from NOAA Ship *Okeanos Explorer.*

Figure 2. *Orpheus* AUV being launched from NOAA ship *Okeanos Explorer* with cameras and lights enabled. *Image credit Art Howard.*

JPL's terrain relative navigation (TRN), used first on NASA's Mars 2020 *Perseverance* rover, is a vision-based system for estimating map-relative position. A similar system integrated into the AUVs will one day enable them to quickly and autonomously sense their location relative to the seafloor to avoid hazards and to recognize seafloor features that may be of scientific interest. This system is an evolution from the velocimetry-based approach to navigation used on NASA's *Ingenuity* Mars helicopter.

Full ocean depth-capable AUVs will enable the science community to systematically explore the hadal zone. The hadal zone is one of the most poorly investigated and mysterious habitats on Earth. There are several hadal depth regions within U.S. waters, including the Aleutian Trench, Mariana Trench, and Puerto Rican Trench. While the NOAA Ocean Exploration 2021 Technology Demonstration was not intended to reach hadal depths, this expedition was an important step in developing the capabilities of the AUV's and advancing the goal to enable hadal-depth operations.

2.4 Rationale for eDNA pilot project

The advancement of eDNA science and the incorporation of eDNA into NOAA Ocean Exploration operations is addressed in the NOAA 'Omics Strategic Plan[8]. The Strategic Plan outlines a series of actions for NOAA to accomplish within specific time frames. NOAA Ocean Exploration could provide baseline eDNA data and sampling that contributes to the implementation of the Strategic Plan.

Collection of eDNA samples also broadly fits into the Implementation Plan for the National Strategy for Ocean Mapping, Exploring, and Characterizing the United States Exclusive Economic Zone[9] (NOMEC). eDNA collection falls under Goal #3: Explore and Characterize Priority Areas of the United States EEZ.

Additional details regarding rationale behind water sample collection for eDNA analysis can be found in the "Feasibility Assessment for Environmental DNA," Appendix E of the Exploration Variables Identified by the NOAA Office of Ocean Exploration and Research[10] (Egan et al., 2021).

2.5 Objectives

The expedition addressed scientific and technology themes and priority areas put forward by the broad ocean science community. The primary objectives of the expedition were to test and develop Orpheus class AUVs, pilot water sampling procedures for eDNA analysis, and survey deepwater areas offshore Florida, Georgia, South Carolina, North Carolina, and Virginia to provide baseline information to support science and management needs. A full

[8] NOAA 'Omics Strategic Plan
[9] Implementation Plan for the National Strategy for Ocean Mapping, Exploring, and Characterizing the United States Exclusive Economic Zone
[10] Exploration Variables Identified by the NOAA Office of Ocean Exploration and Research

rundown of expedition objectives can be found in the Project Instructions: EX-21-02 2021 Technology Demonstration (AUV &Mapping)[11] (White, 2021). The core objectives of this expedition were to:

- Develop launch and recovery procedures for Orpheus class AUVs in coordination with Chief Boatswain, Chief Engineer, Operations Officer, Commanding Officer, and Mission Team.
- Conduct Terrain-Relative Navigation (TRN) calibration using small boats during the first 3-4 days of EX-21-02
- Deploy and test two Orpheus class AUVs
- Test and validate us of TRN software
- Conduct CTD operations while ship is on station with AUVs in the water
- Trigger CTD Niskin bottles at different depths in order to sample a diversity of zones for eDNA sampling
- Draft a standard operating procedures document for the collection and processing of water samples as well as associated sensor and metadata, for the purpose of eDNA analysis.
- Acquire data on deepwater habitats in the southeastern U.S. continental margin to support priority science and management needs.
- Explore U.S. maritime heritage by identifying and investigating sonar anomalies as well as characterizing shipwrecks.
- Collect high-resolution bathymetry in areas with no or low-quality mapping data.
- Engage a broad spectrum of the scientific community and the public in telepresence-based exploration.
- Provide a foundation of publicly accessible data and information products to spur further exploration, research, and management activities.

3. Participants

EX-21-02 included onboard mission personnel as well as shore-based science personnel who supported operations via telepresence. See **Table 1** for the onboard personnel who supported EX-21-02.

[11] doi:10.25923/ksks-3e94

Table 1. EX-21-02 onboard mission team personnel

Name	Title	Affiliation
Michael White	Expedition Coordinator	NOAA Ocean Exploration/Cherokee Federal
Kim Galvez	Expedition Coordinator (training)	NOAA Ocean Exploration/Cherokee Federal
Derek Sowers	Mapping Lead	NOAA Ocean Exploration/Cherokee Federal
Daniel Freitas	Mapping Watch Lead	University Corporation for Atmospheric Research
Jason Meyer	Mapping Watch Lead	University Corporation for Atmospheric Research
Katharine Egan	eDNA Lead	NOAA Ocean Exploration
Meredith Everett	eDNA Subject Matter Expert	NOAA Northwest Fisheries Science Center
Rachel Gulbraa	Engagement Specialist	NOAA Ocean Exploration/ University Corporation for Atmospheric Research (UCAR)
Tim Shank	*Orpheus* Science Lead	Woods Hole Oceanographic Institution
Casey Machado	*Orpheus* Engineer Lead	Woods Hole Oceanographic Institution
Andy Klesh	*Orpheus* Engineer	NASA Jet Propulsion Laboratory
Russell Smith	*Orpheus* Engineer	NASA Jet Propulsion Laboratory
Jessica Kaelblein	Videographer	Inner Space Center/University of Rhode Island
Jim Meyers	Computer Administrator	Global Foundation for Ocean Exploration
Chris Wright	Computer Administrator	Global Foundation for Ocean Exploration
Brian Doros	Video Engineer	Global Foundation for Ocean Exploration
Art Howard	Videographer	Global Foundation for Ocean Exploration

4. Methodology

To accomplish its objectives, EX-21-02 used:

- Two Orpheus class AUVs, *Orpheus* and *Eurydice*
- Vacuum pump and manifold to filter 2L of water per sample through a 0.45 µm filter for later eDNA analysis
- CTD rosette with altimeter, SBE-9plus conductivity sensor (x2), temperature sensor (x2), SBE-43 dissolved oxygen sensor, ECO-FLNTU turbidity sensor, and a Pacific Marine Environmental Laboratory (PMEL) oxygen reduction potential sensor.
- Sonar systems (Kongsberg EM 304 multibeam sonar, Knudsen 3260 sub-bottom profiler, Simrad EK60 and EK80 split-beam sonars, and Teledyne acoustic Doppler current profilers) to conduct mapping operations at night and when the ROVs were on deck.
- A high-bandwidth satellite connection to provide real-time ship-to-shore communications (telepresence).

All environmental data collected by NOAA must be covered by a data management plan to ensure they are archived and publicly accessible. The data management plan for EX-21-02 can be found in the Project Instructions: EX-21-02 2021 Technology Demonstration (AUV & Mapping)[12] (White, 2021).

4.1 Orpheus class AUV operations

The Orpheus class AUVs were packed and shipped from WHOI and staged at the Port Canaveral Commercial Port about a week before departure. Both vehicles and supporting equipment fit into a 20' standard container (**Figure 3**).

[12] doi:10.25923/ksks-3e94

Figure 3. WHOI Engineer Casey Machado in front of AUV shipping container

Both vehicles were staged on the fantail of *Okeanos Explorer*. Power and charging were staged on the port side workbench in the hangar. The WHOI/NASA JPL team set up in the ROV workshop. Several ethernet cables were run from the vehicles on deck and passed through to the workshop to enable communications between engineers in the workshop and the vehicles on deck. This was critical for pre-deployment checklist items. The ethernet cables were disconnected immediately before launch.

During deployment and recovery, the ship was set up in Dynamic Positioning (DP) mode and control of the ship was passed to the Aft-con station, similar to ROV operations. The vehicles were launched using the port side crane which was equipped with a retired spectra line. The use of spectra line was very much preferred by the AUV team and the ship should continue its practice of retaining retired spectra line (which are no longer used for ROV operations after pull tests). In anticipation of possibly completing AUV operations using the starboard crane in the future, it is advised to install spectra on that crane as well. The ship and mission team developed AUV deployment and recovery checklists which are available upon request.

AUV deployments occurred exclusively during daytime hours. The team would arrive on station and evaluate weather, sea state, and forecast to determine if a safe deployment could occur. During later dives, the team attempted to target interesting seafloor features such as potential biogenic coral mounds. Once the ship was on station, the mission team would use data from the ADCPs to evaluate current and determine final ship position prior to deployment. To target features at depth, the mission team would provide a final deployment location to the Bridge after estimating an average AUV descent rate from the surface to the seafloor. This calculation assumed the AUVs were completely submissive to current, and was informed by binned and averaged current data at different depths as obtained from the OS38 ADCP data. After deployment, the mission team would then request the ship move over to the seafloor target location where ultrashort baseline (USBL) range tracking could be reestablished (**Figure 4**). This process took several iterations but was successfully accomplished during the later AUV deployments. Post-dive review of the downward looking video data appeared to validate that the AUVs had indeed piloted missions over the targeted features of interest (e.g., a coral mound feature). The USBL was hung off the ship and not fixed to the hull or a pole. This equipment set up did not support a heading calibration, and thus the USBL was only used for general range measurements from the ship to the AUVs – not for determining the actual coordinates of the vehicles' position.

The team gradually established a viable concept of operations, one of the primary goals of the technology demonstration, to complete daily dive operations by the last several deployments. The team were also able to complete CTD rosette casts while on station over an AUV dive location. Doing additional over-the-side operations is atypical of projects supporting tethered AUVs.

Figure 4. Ultrashort baseline (USBL) software on laptop staged on the grated deck during successful range tracking of *Orpheus* AUV in water.

AUV missions were programmed for four to six hours, depending on engineering and science objectives. The AUVs are equipped with a burn wire and drop weights that trigger after a preprogrammed set of time or after the burn wire expires. The team demonstrated the successful use of a reliable iridium beacon on *Orpheus*. When the AUV surfaces, it sends a series of direct messages (**Figure 5**) to a digital address containing the vehicle's precise latitude and longitude position (actually more decimal places than the ship's navigation system could ingest). These coordinates were continuously supplied to the bridge for highly-efficient set ups for recovery.

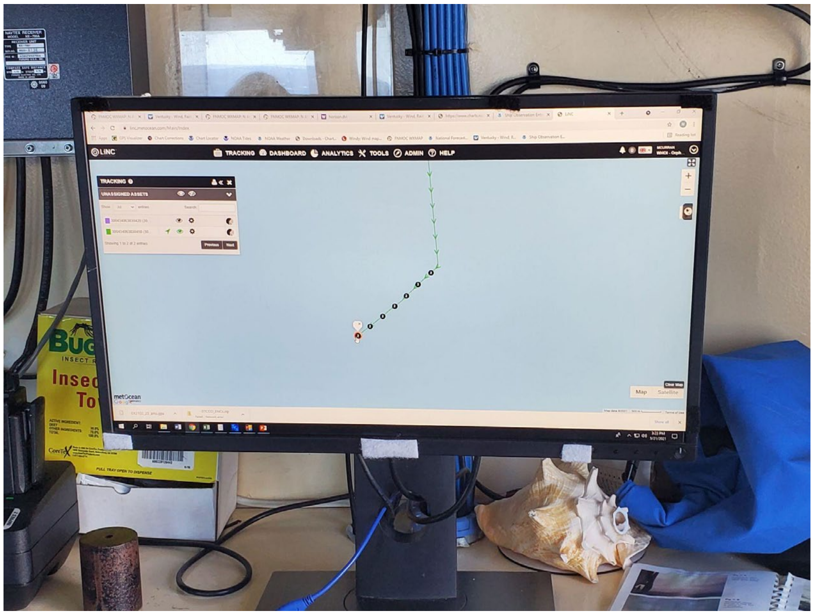

Figure 5. Bridge view of Iridium satellite tracking of surfaced vehicle.

4.1.1 Testing of Analytic Instrument Systems, Inc. (AIS) DLK-ISEA-X chemical sensor

During EX-21-02 the AUVs were also equipped with an AIS BLK-ISEA-X chemical sensor. The instrument utilizes voltammetric scans to produce in-situ chemical measurements. The instrument can detect a variety of reduced chemical species, and can log oxygen, peroxide, sulfide and sulfide species, iron and manganese. This electrochemical sensor uses an array of autonomous self-cleaning electrodes to increase endurance (it can last for a 3-week dive series). The electrodes can collect data in the water column and in sediments. The instrument can also collect pH and temperature data and an auxiliary channel is available for custom sensing. The instrument has multiple ways to communicate and log data.

4.2 eDNA Sampling Operations

During EX-21-02, water column samples were collected using Niskin bottles on the ship's CTD rosette. The primary goal of the sampling operations was to sample for filtering and processing onboard, followed by post-expedition eDNA analysis. Both deepwater (> 200m) and shallow-water CTD casts were conducted. The sampling strategy for the deepwater CTD casts consisted of collecting two samples 5 m from the seafloor, one sample 10 m from the seafloor, and then one sample 20 m from the seafloor. The sampling strategy also included collecting water samples below, within, and above the deep scattering layer (DSL), which was identified using the EK60 sonar. Two water samples were collected within the DSL. One water sample was then collected at the chlorophyll max, at the surface, and the rest of the samples were evenly spaced throughout the water column. Two water samples were pulled from the same Niskin bottle 5 m from the seafloor and within the DSL to identify any variability within samples. For shallow-water casts, two samples were collected 5 m from the seafloor and at the surface. Rather than pulling from the same Niskin, two Niskin bottles were fired and counted as replicate samples for shallow-water casts. The rest of the water samples collected were evenly spaced throughout the water column. For each water sample collected, date, time, latitude, longitude, depth, salinity, temperature, dissolved oxygen, oxygen reduction potential, turbidity and fluorescence were recorded at the time of collection using a Scientific Computer System (SCS) event.

Water filtration procedures were based off of Everett and Park (2018). The Niskin bottles on the ship's CTD rosette hold 12L of water, but 2L of water were filtered per sample using a 0.45 µm filter to capture the DNA. The filter was then stored using Longmire's buffer solution, which keeps the DNA stable at room temperature until the samples can be processed (Longmire et al., 1997). For every CTD cast conducted, one tap water, or blank, sample was also filtered per cast to detect any possible background contamination.

During EX-21-02 a detailed Standard Operating Procedures document for eDNA sample collection during NOAA Ocean Exploration operations on the NOAA Ship *Okeanos Explorer* was developed and can be found in Appendix B of this report. The SOP details sample processing procedures, strategy, and data management.

4.3 Acoustic Operations

Acoustic operations included Kongsberg EM 304 multibeam, Simrad EK60 and EK80 split-beam, Knudsen sub-bottom profiler, and acoustic Doppler current profiler (ADCP) data collection. The NOAA Ship *Okeanos Explorer* Survey Readiness Report 2021[13] (Candio et al., 2021) details the acoustic mapping hardware and software capabilities of NOAA Ship *Okeanos Explorer*, and the performance evaluations undertaken by NOAA Ocean Exploration in preparation for the 2021 field season. For further information about general

[13] doi:10.25923/qbjz-m470

equipment calibration procedures, data acquisition, processing, reporting, and archiving see the NOAA OER Deepwater Exploration Mapping Procedures Manual V1[14] (Hoy et al., 2020), available in the NOAA Central Library and from the NOAA Ocean Exploration website.

4.3.1 Multibeam Sonar (Kongsberg EM 304)

Multibeam seafloor mapping data were collected using the Kongsberg EM 304 sonar, which operates at a frequency of 30 kHz. Multibeam mapping operations were conducted during all overnight transits between ROV dive sites. Multibeam data quality was monitored in real time by acquisition watchstanders. Ship speed was adjusted to maintain data quality as necessary. For more detailed information about the EM 304 MKII multibeam echosounder acceptance testing and calibration procedures, see the NOAA Ship *Okeanos Explorer* EX-21-01 EM 304 MKII Sonar Acceptance and Testing Report[15] (Candio et al., 2021)

Whenever possible, transits were designed to maximize coverage over seafloor areas with no previous high-resolution mapping data. In these focus areas, line spacing was generally planned to ensure 30% overlap between lines at all times. Cutoff angles in the Seafloor Information System (SIS) software were generally adjusted on both the port and starboard sides to ensure the best balance between data quality and coverage. Overnight surveys were also completed in areas that were previously mapped with a lower resolution multibeam sonar system.

These operations collected data on seafloor depth (bathymetry), seafloor acoustic reflectivity (seafloor backscatter), and water column reflectivity (water column backscatter). Mapping operations took place during overnight transits and whenever the AUVs were on deck. Lines were planned to maximize edge matching of existing data or filling of data gaps in areas with incomplete bathymetry coverage. In regions with no existing data, exploration transit lines were planned to optimize potential discoveries. Focused mapping operations were completed east of Port Canaveral, Florida, and on the Blake Plateau, where no or little modern multibeam data exists. Targeted underwater cultural heritage mapping surveys were conducted at 4-8 knots (depth dependent; slower at deeper depths to increase sounding density and potential for shipwreck discovery) off the coast of North Carolina and Virginia to search for potential shipwrecks as requested by the Monitor National Marine Sanctuary.

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[14] doi:10.25923/jw71-ga98

[15] doi:10.25923/5fm9-0f17

4.3.2 Sub-Bottom Profiler (Knudsen Chirp 3260)

The primary purpose of the Knudsen Chirp 3260 (3.5 kHz) sonar is to image sediment layers underneath the seafloor to a maximum depth of about 80 m below the seafloor, depending on the specific sound velocity of the substrate. The sub-bottom profiler was operated simultaneously with the multibeam sonar during mapping operations to provide supplemental information about the sedimentary features underlying the seafloor. On EX-21-02 generally the sub-bottom profiler was not run in areas shallower than 50 m.

4.3.3 Split-Beam Sonars (Simrad EK60 and EK80)

Okeanos Explorer is equipped with five split-beam transducers, three Simrad EK60 general purpose transceivers and two Simrad EK80 wideband transceivers. The frequencies of the EK60 are 18, 38, 120, and 200 kHz. The frequency of the EK80 is 70 kHz. During EX-21-02 all EK sonars were run in narrowband mode. For detailed information about the calibration procedures and results of the Simrad EK60/80 echosounders see the 2021 EK60/80 Calibration Report[16] (Copeland et al., 2021).

These sonars were used continuously throughout EX-21-02 during both overnight mapping operations and daytime AUV/CTD operations. The sonars provided calibrated target strength measurements of water column features such as dense biological layers, schools of fish, and gaseous seeps. EK60 and EK80 data were used during CTD rosette operations to target Niskin bottle firing with respect to deep scattering layers, which are aggregations of biological organisms in the water column.

4.3.4 Acoustic Doppler Current Profiler (Teledyne Workhorse Mariner and Teledyne Ocean Surveyor ADCPs)

Okeanos Explorer is equipped with two ADCPs: a Teledyne Workhorse Mariner (300 kHz) and a Teledyne Ocean Surveyor (38 kHz). The ADCPs provide information on the speed and direction of currents underneath the ship. They were used during AUV deployments and CTD casts to support safe deployment and recovery of the vehicles and to provide ancillary data for eDNA sampling. The ADCPs were not used during multibeam mapping due to sonar interference with the EM 304.

During EX-21-01 the 38 kHz transducer experienced temperature spikes indicating possible water intrusion and imminent failure. The ADCP was used during EX-21-02 to help assess currents at AUV dive sites, with the data appearing reasonable. However, the data from

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[16] doi:10.25923/v5kz-ge28

this expedition for the 38 kHz ADCP is of unknown quality given the expected degradation of the transducer from water intrusion.

4.3.5 Expendable Bathythermograph (XBT) Systems

Expendable bathythermographs (XBTs) were collected every six hours or more often as dictated by oceanographic conditions and applied in real time using Sound Speed Manager and SIS. Sound speed at the sonar head was determined using sound speed from a flow-through thermosalinograph (TSG).

4.4 Conductivity, Temperature, and Depth

Conductivity, temperature, and depth measurements were collected via a dedicated CTD rosette lowered with a winch. Additional sensors installed on the CTD rosette included measured light scattering (LSS), dissolved oxygen (DO), fluorescence (as a proxy for chlorophyll) and oxygen reduction potential (ORP).

4.5 Sun Photometer Measurements

NOAA Ocean Exploration gathers limited at-sea measurements aboard *Okeanos Explorer* to support a NASA-led, long-term research effort that assesses marine aerosols. As time allowed on cloud-free days, onboard personnel collected georeferenced sun photometer measurements for the Maritime Aerosol Network (MAN) component of the Aerosol Robotic Network (AERONET). AERONET is a network of sun photometers that measure atmospheric aerosol properties around the world. MAN complements AERONET by conducting sun photometer measurements on ships of opportunity to monitor aerosol properties over the global ocean.

5. Clearances and Permits

Pursuant to the National Environmental Policy Act (NEPA), NOAA Ocean Exploration is required to include in its planning and decision-making processes appropriate and careful consideration of the potential environmental consequences of actions it proposes to fund, authorize, and/or conduct. The companion manual for NOAA Administrative Order 216-6A describes the agency's specific procedures for NEPA compliance.

An environmental review memorandum was completed for NOAA Ocean Exploration expeditions on *Okeanos Explorer* in 2021 in accordance with Section 4 of the companion manual in the form of a categorical exclusion worksheet. Based on this review, a categorical exclusion was determined to be the appropriate level of NEPA analysis necessary, as no extraordinary circumstances exist that require the preparation of an environmental assessment or environmental impact statement. NOAA Ocean Exploration is preparing a programmatic environmental assessment to cover future expeditions.

As required under Section 7 of the Endangered Species Act (ESA), NOAA Ocean Exploration conducted an informal consultation with NOAA's National Marine Fisheries Service (NMFS) Office of Protected Resources to request their concurrence with NOAA Ocean Exploration's biological evaluation determining that NOAA Ocean Exploration's operations on *Okeanos Explorer* conducted in 2021 may affect, but are not likely to adversely affect, ESA-listed marine species. In a Letter of Concurrence dated February 3, 2021, the chief of the ESA Interagency Cooperation Division in the NMFS Office of Protected Resources wrote that NMFS concurs with NOAA Ocean Exploration's determination.

In addition, NOAA Ocean Exploration consulted with the NMFS Greater Atlantic Fisheries Office (GARFO) on potential impacts of operations to essential fish habitat (EFH) in the greater Atlantic region under the Magnuson-Stevens Fishery Conservation and Management Act. NOAA Ocean Exploration received a letter of acknowledgement from GARFO on March 10, 2021, that covers expedition activities from April 1, 2021 until September 31, 2021.

Appendix A contains a copy of the NEPA Categorical Exclusion. The Endangered Species Act (ESA) Section 7 Letter of Concurrence and a Letter of Acknowledgement from the Greater Atlantic Regional Fisheries Office (GARFO) are in the "NOAA Ship *Okeanos Explorer* FY2021 Field Season Instructions."

NOAA Ocean Exploration supports the standards for conducting marine archaeological activities enumerated in the Annex Rules of the UNESCO Convention on the Protection of Underwater Cultural Heritage.

6. Schedule and Map

EX-21-02 was a total of 14 days at sea, from May 14 to May 27, 2021. It departed from Cape Canaveral, Florida, and returned to port in Norfolk, Virginia. See **Table 2** for a day-by-day breakdown of EX-21-02. See **Figure 6** for a map of EX-21-02's track, AUV deployments, CTD rosette casts, and bathymetry collected.

*Note, the WHOI/NASA JPL AUV team counts Orpheus class AUV dives consecutively across expeditions. Thus, the *first* AUV deployment of EX-21-02 was *Orpheus004*. For consistency, the AUV dive numbers are recorded the same way in this report. In contrast, NOAA Ship *Okeanos Explorer* operations, including CTD casts, are counted consecutively starting at 01 on each cruise.

Table 2. EX-21-02 schedule

Date (UTC)	Activity
5/11	Mobilization Port Canaveral, Florida. AUV 20' ft container staged next to ship. Mission team moved onboard.
5/12	Mobilization Port Canaveral, Florida.
5/13	Mobilization Port Canaveral, Florida. Walkthrough of AUV Launch and Recovery.
5/14	1000 Depart Port Canaveral, Florida. Tethered launch and recovery of *Orpheus* for engineering testing. Overnight Mapping
5/15	*Orpheus* deployment 004*, shallow dive, 'Port Canaveral East'. Small boat calibration operations. CTD rosette cast 001. Overnight Mapping.
5/16	*Orpheus* dive 005 and CTD cast 002, both shallow about 4 nautical miles from Port Canaveral. Overnight transit mapping operations
5/17	*Orpheus* dive 006, first deep dive (860 m). CTD cast 003 completed with *Orpheus* in the water. Overnight Blake Plateau mapping.
5/18	*Orpheus* dive 007. CTD cast 004. Overnight mapping operations on the Blake Plateau.
5/19	*Orpheus* dive cancelled due to weather. CTD cast 005 in the main axis of the Gulf Stream. 24-hour mapping operations on the eastern Blake Plateau.
5/20	Inaugural dive with AUV *Eurydice* in shallow (30 m) water. CTD cast 006. Overnight mapping operations.
5/21	Orpheus deployment 008. CTD cast 007. Overnight mapping operations.
5/22	*Orpheus* dive cancelled, engineering and maintenance day. CTD Cast 008. 24 hour mapping operations on the Blake Plateau.
5/23	*Orpheus* dive 009. CTD cast 009. Overnight mapping operations on the Blake Plateau.
5/24	*Orpheus* dive 010. Final AUV dive. Overnight mapping operations on the Blake Plateau.
5/25	CTD cast 011. Begin northward transit, overnight transit mapping operations.
5/26	Attempted deployment of a small ROV brought onboard by NASA to attempt to image wreck site. CTD cast 012. Mapping operations in support of Monitor National Marine Sanctuary (MNMS).
5/27	Arrival in Norfolk, Virginia. Demobilization. Unloading of 20' container.

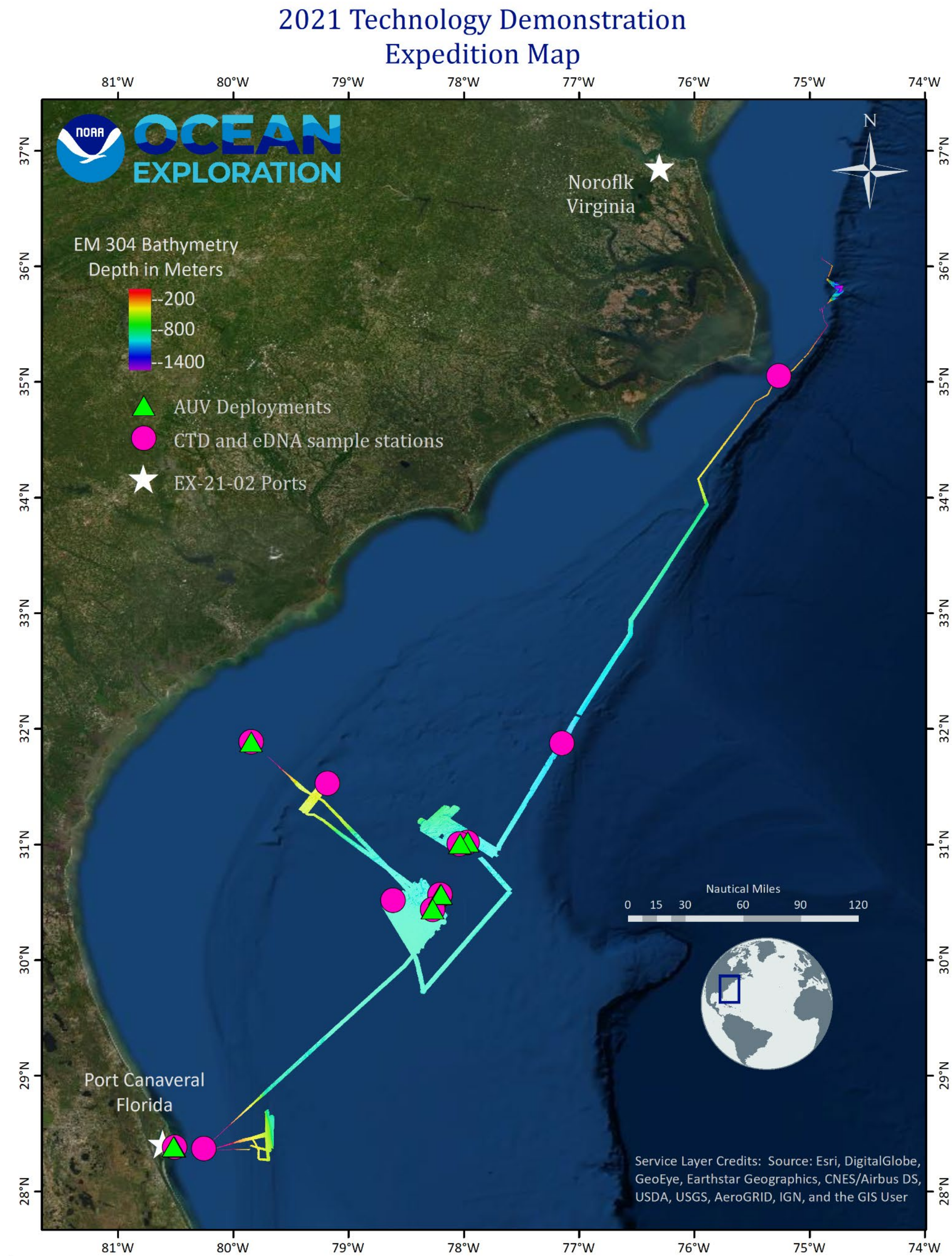

Figure 6. Map showing EX-21-02's track, 8 AUV deployment dive sites, 12 CTD casts and bathymetry data collected.

7. Results

Metrics for EX-21-02's major exploration and scientific accomplishments are summarized in **Table 3**. More detailed results are presented in the subsections that follow.

Table 3. Summary of exploration metrics for EX-21-02

Exploration Metrics	Totals
Days at sea	14
Days at sea in U.S. waters	14
Linear km mapped by EM 304	3,345
Square km covered by EM 304	9,669
Square km covered by EM 304 in U.S. waters deeper than 200m	9,655
Vessel CTD casts	12
Water Samples collected for eDNA	120
XBT casts	83
AUV deployments	8
AUV deployments in U.S. waters	8
Maximum ROV seafloor depth (m)	866
Minimum ROV seafloor depth (m)	12
Total time on bottom (hh:mm:ss)	16:14:00
Total AUV time (hh:mm:ss)	32:59:00

7.1 AUV Survey Results

Depth ranges explored during the 8 AUV (**Figure 7**) deployments were between 12 and 866 m. During the 8 dives, the AUVs spent a total of 16:40 hours on the bottom and 32:59 hours in the water. See **Table 4** for deployment-specific information.

Table 4. Summary information for the 8 AUV dives conducted during EX-21-02. The WHOI/NASA JPL AUV team counts Orpheus class AUV dives consecutively across expeditions. Thus, the *first* AUV deployment of EX-21-02 was *Orpheus004*. For consistency, the AUV dive numbers are recorded the same way in this report.

Dive #	Site Name	Date (local) (yyyymmdd)	Deployment Latitude (dd)	Deployment Longitude (dd)	Max Depth (m)	Dive Duration (hh:mm:ss)	Dive objectives
Orpheus 004	Port Canaveral East	20210515	28.38146	-080.51023	12	3:15:00	Heading testing, TRN calibration
Orpheus 005	Port Canaveral East 02	20210516	28.38775	-080.51698	13	3:00:00	Heading testing, USBL testing
Orpheus 006	Central Blake Plateau	20210517	30.56536	-078.20643	860	3:54:00	First deepwater dive, USBL tracking testing, scientific equipment testing.
Orpheus 007	Central Blake Plateau 02	20210518	30.56893	-078.19854	840	6:24:00	Longer duration dive, programmed longer linear transects.
Eurydice 001	East Savannah	20210520	31.886759	-079.84461	37	1:45:00	Vehicle's inaugural dive. Testing of electronics, general readiness.
Orpheus 008	Central Blake Plateau 03	20210521	30.44450	-078.27111	800	6:18:00	Continued testing of vehicles ability to drive preprogrammed missions, continued 'lawn mowing' patterns. After the dive the team discovered vertical thrusters had an issue and the entire dive was completed in the water column.
Orpheus 009	Central Blake Plateau 04	20210523	31.02293	-077.96640	860	5:06:00	Continued to monitor battery draw. Vehicle was able to fly on and off coral mounds. Adjusted bottom tracking to fly closer to the seafloor.
Orpheus 010	Central Blake Plateau 05	20210524	31.00778	-078.03363	866	3:17:00	Readjusted vehicle altitude to fly closer to the seafloor. Successfully addressed battery discharged encountered on earlier dives.

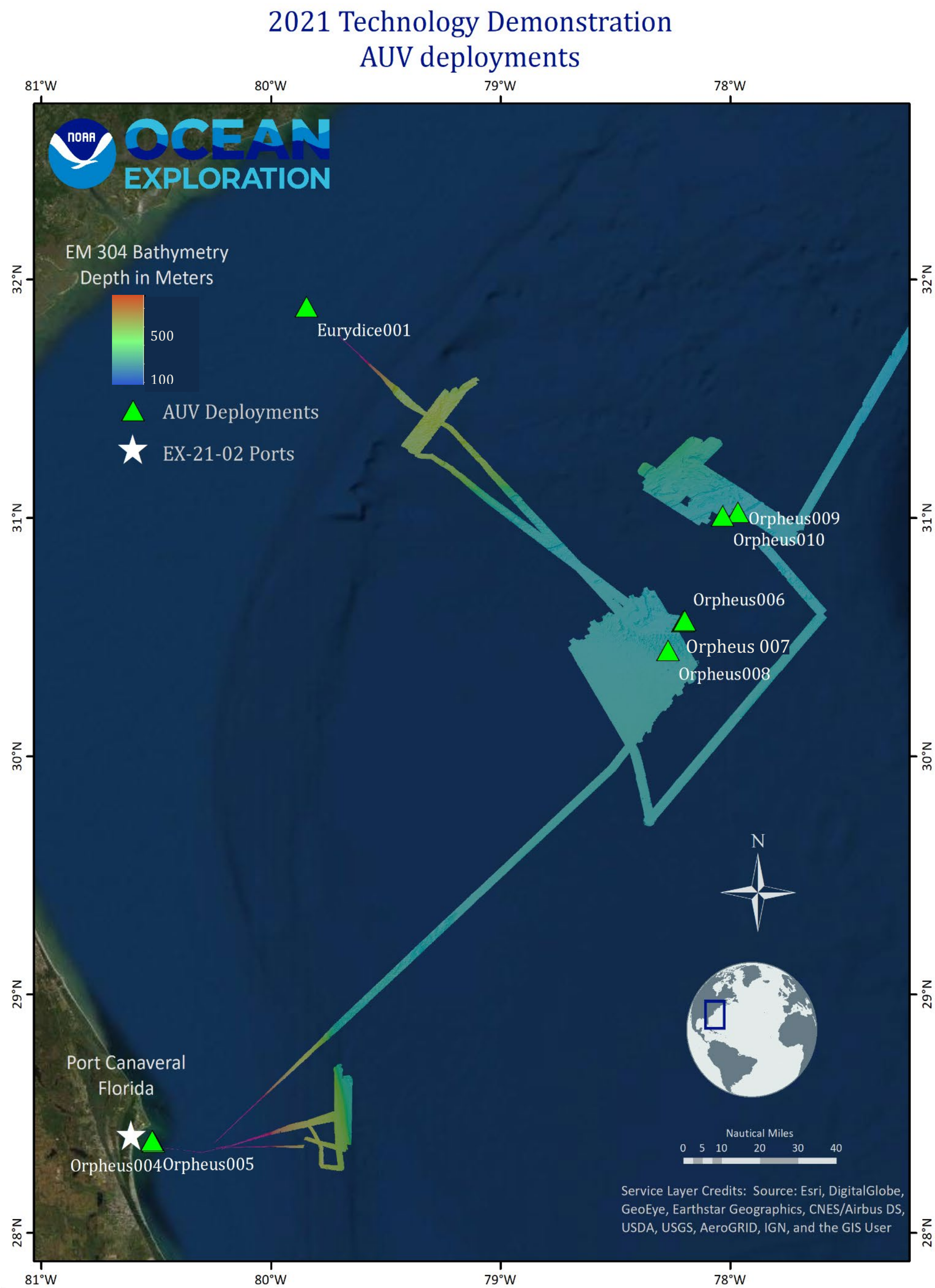

Figure 7. AUV deployments and EM 304 bathymetry from EX-21-02.

As described earlier in this report, the mission team was able to develop a synergistic method for AUV/CTD/mapping operations during EX-21-02. Similar to the way multibeam, ADCP, and other data collected in real-time is used to plan ROV dives, the mission team was able to transfer those processes to plan and execute AUV dives.

During the eight AUV deployments the WHOI/NASA JPL team was able to demonstrate the vehicle's ability to follow the programmed missions. *Orpheus* conducted lawn mowing patterns of different lengths and line spacing, diagonal connecting lines, and the team tested on complex upward and downward slopes to demonstrate bottom tracking. The team also improved software capabilities for more robust vehicle heading and altitude controls. This improved the maneuverability of the vehicles and enabled them to control the altitude above the seafloor within centimeters of accuracy.

The later AUV deployments targeted scientific features of interest, namely mound features confirmed to be covered in coral rubble and living coral, on the Blake Plateau. **Figures 8 - 10** display representative images from the 4K video collected during *Orpheus* deployments. These include living coral colonies, sponges, coral rubble, ripples, and water column imagery.

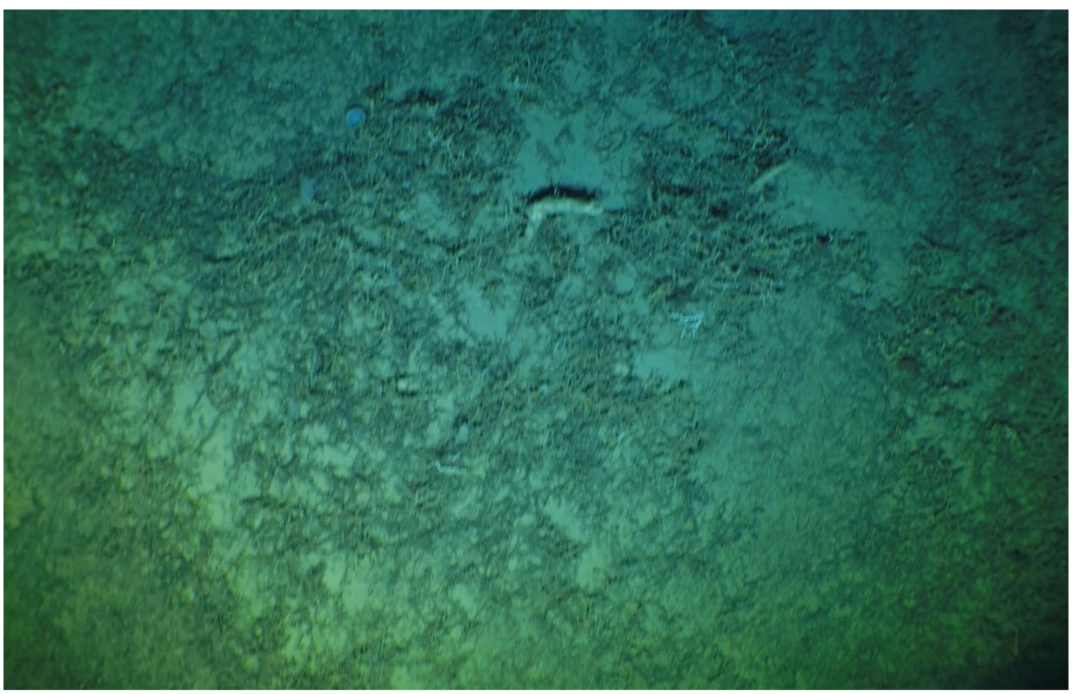

Figure 8. Example of living sponges and coral rubble from *Orpheus* 009.

Figure 9. Sand ripples and coral rubble observed on *Orpheus* 010.

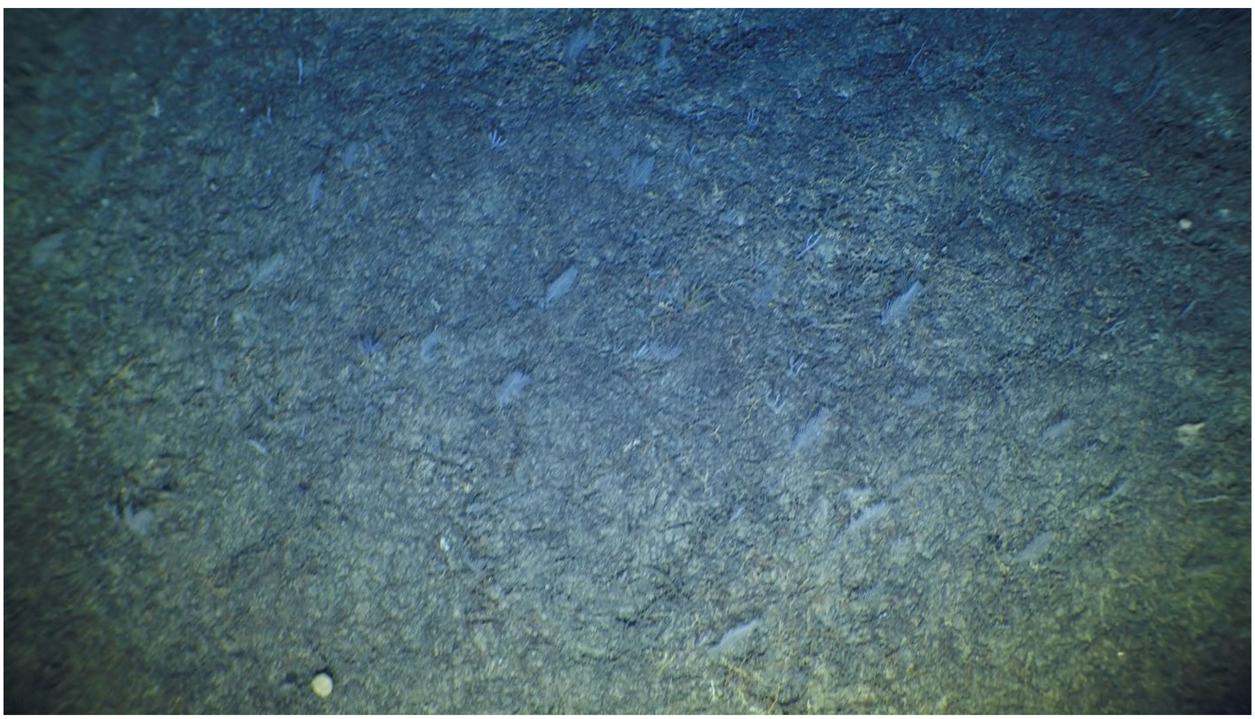

Figure 10. Over a dozen sponges as well as living coral observed on *Orpheus* 010.

The WHOI/NASA JPL teams acquired key visual data to advance the next engineering steps for TRN, a core objective of the expedition and a major step for NASA JPL. During the expedition, the imagery was put into the TRN software, which identified and followed thousands of unique points in real time during playback that it followed for feature recognition.

During EX-21-02, the team collected the first autonomous in-situ electrochemical sensing via an AIS ISEA-X instrument, obtaining 1,624 chemical scans from near bottom and water column, and sending data from the instrument to the AIS ISEA-X shore-side lab via dedicated WiFi in less than 20 minutes after the dive (detecting reduced and oxidized chemical species, oxygen, peroxide and others). This instrument is being developed for *Orpheus* class AUVs not only for characterizing water column properties throughout the water column (e.g. oxygen minimum zones) and near bottom chemistry, but also for locating chemosynthetic environments in the hadal zone.

The mission team discovered (albeit through an unintended battery issue) that *Orpheus* is capable of conducting missions and gathering imagery solely in the water column, using depth data and flying pre-planned patterns at specific depths. Such a capability could be leveraged in the future towards water column exploration and characterization and could mean the Orpheus AUVs can function as a benthic and water column exploration AUV during a single expedition.

Through analysis of vehicle data during the expedition the team found Orpheus AUVs are capable of moving at relatively high speeds over the seafloor. During an average two hours of bottom time, the vehicles were covering an estimated four kilometers using only 50% thrusts, all while gathering 4K imagery of seafloor. If the team was able to incorporate geodetic tracking through a USBL or other means, the vehicles also have the potential to extensively ground truth multibeam bathymetry and backscatter. Such an ability could permit greater efficiency in deep habitat assessment and deep sea mineral identification.

7.1 CTD Rosette Results

A total of 12 vertical casts were conducted. Of the deep water casts, only one cast (CTD005) did not use the water sample collection strategy outlined in the Methodology section. **Table 6** contains CTD cast information and **Figure 11** displays the CTD cast locations.

Table 6. CTD rosette cast information

CTD Name	CTD Number	Date (UTC)	Deployment Time (UTC)	Deployment Latitude (decimal degrees)	Deployment Longitude (decimal degrees)	Recovery Time (UTC)	Recovery Latitude (decimal degrees)	Recovery Longitude (decimal degrees)	Max Depth (meter)
EX2102_CTD001_20210515	CTD001	15May2021	10:26:49	28.38146	-80.51023	10:32:57	28.38146	-80.5102	7.729
EX2102_CTD002_20210516	CTD002	16May2021	19:00:27	28.36838	-80.2567	19:10:21	28.36838	-80.2567	25.516
EX2102_CTD003_20210517	CTD003	17May2021	16:23:43	30.56209	-78.20693	17:22:24	30.56209	-78.2069	859.16
EX2102_CTD004_20210518	CTD004	18May2021	16:25:23	30.56503	-78.20693	17:23:26	30.5598	-78.2067	860.34
EX2102_CTD005_20210519	CTD005	19May2021	13:06:11	31.52878	-79.18552	14:07:16	31.56185	-79.1478	525.54
EX2102_CTD006_20210520	CTD006	20May201	12:23:28	31.88705	-79.84402	12:33:54	31.88705	-79.8442	33.466
EX2102_CTD007_20210521	CTD007	21May2021	15:32:58	30.43702	-78.27061	16:32:23	30.43702	-78.2706	817.71
EX2102_CTD008_20210522	CTD008	22May2021	13:42:51	30.51721	-78.61515	14:38:45	30.51717	-78.6151	812.56
EX2102_CTD009_20210523	CTD009	23May2021	15:59:53	31.01711	-77.97306	16:54:30	31.01711	-77.9730	858.03
EX2102_CTD010_20210524	CTD010	24May2021	16:08:34	31.00296	-78.03873	17:26:41	31.00297	-78.0387	834.47
EX2102_CTD011_20210525	CTD011	25May2021	13:25:27	31.87544	-77.15023	14:41:32	31.87544	-77.1502	1056.8
EX2102_CTD012_20210526	CTD012	26May2021	14:20:31	35.05326	-75.2676	14:29:29	35.05326	-75.2676	70.745

2021 Technology Demonstration
CTD and water sample stations

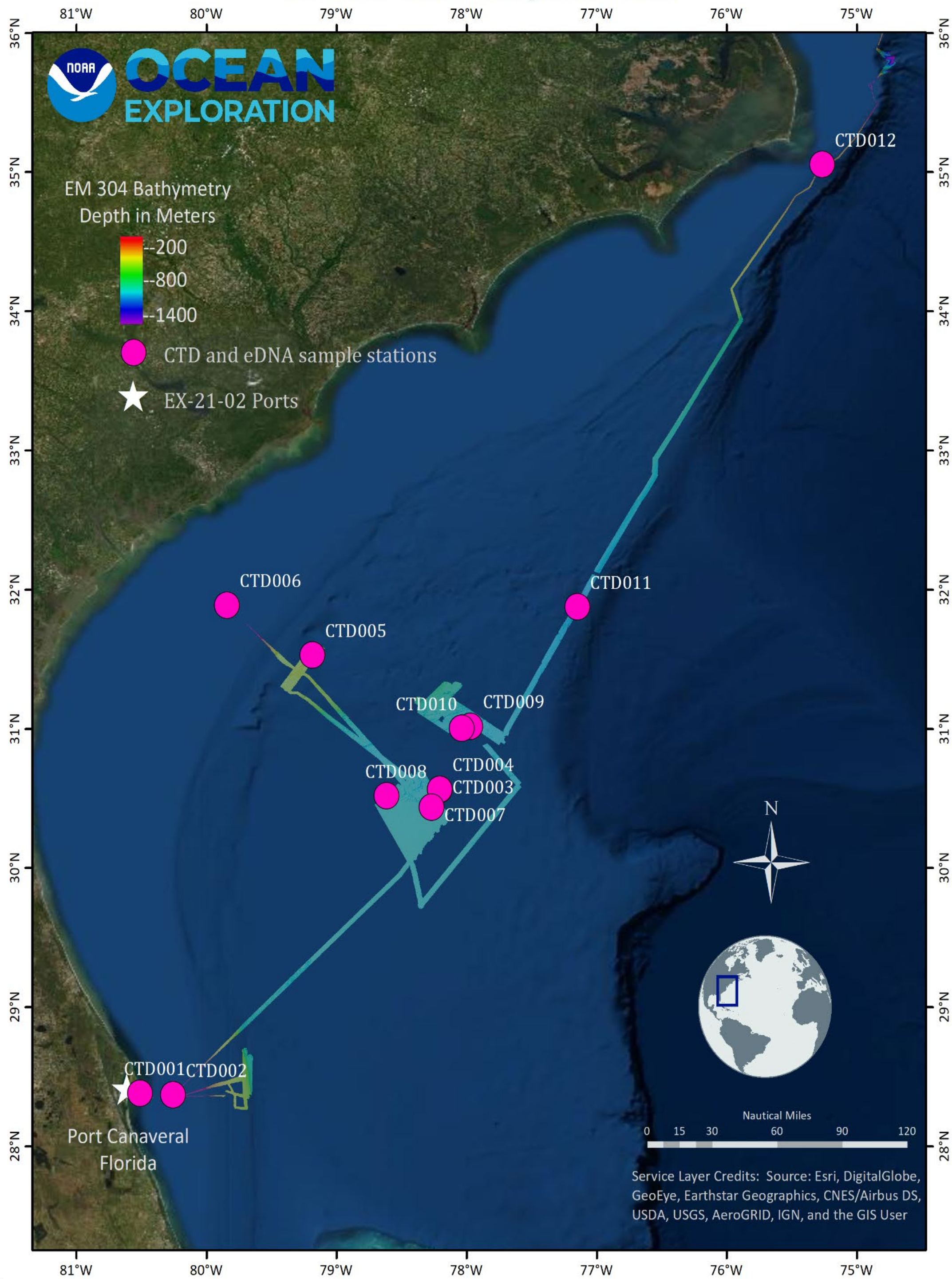

Figure 11. EX-21-02 CTD cast location and bathymetry.

7.1.1 Accessing Data

NCEI Oceanographic Dataset

The EX-21-02 NCEI archival dataset contains oceanographic data collected from NOAA Ship *Okeanos Explorer* in the North Atlantic Ocean from 2021-05-14 to 2021-05-27. Data acquisition from shipboard sensors includes navigational data and meteorological data (altimetry, conductivity, depth, echo sounder, fluorescence, oxygen, pressure, relative humidity, salinity, sound velocity, temperature, turbidity, water temperature, wind, wind speed). Additional data includes profile data (ASVP, CTD, and XBT) and the completed CTD summary forms that describe CTD operations and metadata. These data can be cited as: Sowers, Derek; White, Michael (2021). Oceanographic data collected during the EX2102: 2021 Technology Demonstration (AUV & Mapping) expedition on NOAA Ship OKEANOS EXPLORER in the North Atlantic Ocean from 2021-05-14 to 2021-05-27 (NCEI Accession 0237317). NOAA National Centers for Environmental Information. Dataset. https://doi.org/10.25921/b5y7-fk33. Accessed [date].

7.2 Sampling Operations Results

A total of 120 water column samples were collected and processed for post-expedition eDNA analysis during EX-21-02. In addition to the water column samples, one control sample (a blank or freshwater sample) was run for each CTD cast, resulting in 12 additional samples. One air sample (i.e., a filter left exposed to the air for one day to detect background contamination) was also collected. Samples were mailed to the NOAA Northwest Fisheries Science Center for DNA extraction and sequencing. See **Table 7** for more cumulative results.

Table 7. Inventory and sensor data from CTD Rosette water samples collected during EX-21-02

CTD#	Sample Name	Date	Time (UTC)	Depth (m)	Temperature (°C)	Salinity (PSU)	Dissolved Oxygen [mg/l]	Oxygen Reduction Potential (mV)	Density (kg/m^3)	Fluorescence (mg/m^3)	Turbidity (NTU)
CTD001	EX2102_CTD001_B01_S01	15May2021	10:30:00	7.452	24.9792	35.626	6.4381	229.6703297	23.8531	NA	NA
CTD001	EX2102_CTD001_B02_S01	15May2021	10:30:14	7.952	24.9834	35.6261	6.4414	229.4261294	23.8541	NA	NA
CTD001	EX2102_CTD001_B03_S01	15May2021	10:31:03	2.699	24.9872	35.6273	6.4411	227.7167277	23.831	NA	NA
CTD001	EX2102_CTD001_B04_S01	15May2021	10:31:13	3.08	24.9844	35.627	6.4287	227.4725275	23.8333	NA	NA
CTD002	EX2102_CTD002_B01_S01	5/16/2021	19:03:40	25.894	24.3591	35.8834	6.4045	192.5518926	24.3155	3.8216	0.9684
CTD002	EX2102_CTD002_B02_S01	5/16/2021	19:03:48	25.568	24.3457	35.8785	6.4166	192.5518926	24.3144	3.8472	0.7608
CTD002	EX2102_CTD002_B03_S01	5/16/2021	19:05:24	14.758	24.4897	35.9003	6.7814	191.5750916	24.2406	1.5395	0.3701
CTD002	EX2102_CTD002_B04_S01	5/16/2021	19:05:33	14.841	24.4953	35.9026	6.7698	191.5750916	24.241	1.5652	0.3579
CTD002	EX2102_CTD002_B05_S01	5/16/2021	19:06:49	3.16	24.5115	35.9076	6.7754	190.8424908	24.1892	0.8728	0.3579
CTD002	EX2102_CTD002_B06_S01	5/16/2021	19:06:57	3.162	24.52	35.907	6.7737	190.5982906	24.1862	0.9498	0.3701
CTD003	EX2102_CTD003_B01_S01	5/17/2021	16:45:46	859.876	8.8058	35.1732	4.4141	189.6214896	31.1947	0.1036	0.1991
CTD003	EX2102_CTD003_B01_S02	5/17/2021	16:45:46	859.876	8.8058	35.1732	4.4141	189.6214896	31.1947	0.1036	0.1991
CTD003	EX2102_CTD003_B02_S01	5/17/2021	16:47:24	849.821	9.2688	35.1902	4.2473	190.1098901	31.0798	0.1549	0.2113
CTD003	EX2102_CTD003_B03_S01	5/17/2021	16:48:41	840.091	9.5229	35.2235	4.2326	190.1098901	31.0155	0.1293	0.2113
CTD003	EX2102_CTD003_B04_S01	5/17/2021	16:53:25	699.618	12.9621	35.6406	4.1146	189.6214896	30.0264	0.1036	0.2113
CTD003	EX2102_CTD003_B05_S01	5/17/2021	16:56:00	650.32	14.4163	35.8963	4.7186	189.1330891	29.6844	0.1036	0.2113
CTD003	EX2102_CTD003_B06_S01	5/17/2021	16:59:24	550.784	16.5276	36.2397	5.0923	188.4004884	29.0123	0.078	0.2113
CTD003	EX2102_CTD003_B06_S02	5/17/2021	16:59:24	550.784	16.5276	36.2397	5.0923	188.4004884	29.0123	0.078	0.2113
CTD003	EX2102_CTD003_B07_S01	5/17/2021	17:02:44	451.178	17.8054	36.4356	5.2491	188.1562882	28.407	0.078	0.1991
CTD003	EX2102_CTD003_B09_S01	5/17/2021	17:09:19	250.661	19.5423	36.6896	5.7099	188.4004884	27.2715	0.0523	0.1869

CTD003	EX2102_CTD003_B10_S01	5/17/2021	17:12:35	152.074	20.8821	36.7975	6.0044	188.1562882	26.5602	0.078	0.1991
CTD003	EX2102_CTD003_B11_S01	5/17/2021	17:14:22	121.491	21.8171	36.8425	6.2781	187.9120879	26.2002	0.9498	0.2113
CTD003	EX2102_CTD003_B12_S01	5/17/2021	17:20:15	2.838	24.6343	36.7524	6.5942	185.2258852	24.7901	1.3344	-0.0695
CTD004	EX2102_CTD004_B01_S01	5/18/2021	16:45:58	859.882	9.316	35.6307	3.8358	197.1916972	31.4588	0.6421	-0.0207
CTD004	EX2102_CTD004_B01_S02	5/18/2021	16:45:58	859.882	9.316	35.6307	3.8358	197.1916972	31.4588	0.6421	-0.0207
CTD004	EX2102_CTD004_B02_S01	5/18/2021	16:47:27	850.616	9.6159	34.9872	3.3524	197.6800977	30.8624	0.6421	-0.0084
CTD004	EX2102_CTD004_B03_S01	5/18/2021	16:48:40	840.486	9.7084	35.0394	2.9824	197.9242979	30.8403	0.6164	-0.0084
CTD004	EX2102_CTD004_B04_S01	5/18/2021	16:51:44	749.502	11.6067	33.9204	3.4774	197.9242979	29.2015	0.5908	-0.0084
CTD004	EX2102_CTD004_B05_S01	5/18/2021	16:54:48	649.902	14.6091	34.23	3.2342	196.7032967	28.3593	0.5908	-0.0329
CTD004	EX2102_CTD004_B06_S01	5/18/2021	16:59:19	500.356	17.0023	35.2706	2.6029	195.970696	27.9327	0.5908	-0.0207
CTD004	EX2102_CTD004_B06_S02	5/18/2021	16:59:19	500.356	17.0023	35.2706	2.6029	195.970696	27.9327	0.5908	-0.0207
CTD004	EX2102_CTD004_B07_S01	5/18/2021	17:02:56	400.821	18.5469	35.9302	2.7698	195.4822955	27.6105	0.5908	-0.0329
CTD004	EX2102_CTD004_B08_S01	5/18/2021	17:05:34	351.05	19.0775	36.3039	2.7827	195.4822955	27.5395	0.5652	-0.0207
CTD004	EX2102_CTD004_B09_S01	5/18/2021	17:08:48	250.491	19.8911	36.2439	2.9661	195.7264957	26.8379	0.5652	-0.0329
CTD004	EX2102_CTD004_B10_S01	5/18/2021	17:12:17	152.006	21.4261	36.2729	3.0578	195.7264957	26.01	0.5395	-0.0329
CTD004	EX2102_CTD004_B11_S01	5/18/2021	17:15:44	66.997	23.7434	35.9166	3.1482	194.993895	24.7028	0.5652	0.5532
CTD004	EX2102_CTD004_B12_S01	5/18/2021	17:20:29	3.93	25.2588	36.4083	3.2197	192.5518926	24.3436	0.5395	-0.0329
CTD005	EX2102_CTD005_B01_S01	5/19/2021	13:37:21	514.704	8.4326	35.0397	3.8741	213.7973138	29.5904	0.2691	-0.0434
CTD005	EX2102_CTD005_B02_S01	5/19/2021	13:44:54	302.579	13.1347	35.6906	4.074	212.3321123	28.2576	0.2691	-0.0177
CTD005	EX2102_CTD005_B03_S01	5/19/2021	13:49:53	203.822	17.0651	36.3044	4.6231	210.6227106	27.4018	0.2569	-0.0434
CTD005	EX2102_CTD005_B04_S01	5/19/2021	13:53:45	103.518	22.8944	36.3914	6.391	207.2039072	25.4706	0.318	1.3925
CTD005	EX2102_CTD005_B05_S01	5/19/2021	13:57:06	14.287	27.3567	36.3482	6.231	204.029304	23.6803	0.2813	-0.0434
CTD006	EX2102_CTD006_B01_S01	5/20/2021	12:26:52	33.575	23.2132	36.1621	6.8107	230.8913309	24.8994	0.2691	0.1874
CTD006	EX2102_CTD006_B02_S01	5/20/2021	12:27:02	33.463	23.214	36.1624	6.8029	230.8913309	24.899	0.2813	0.2387
CTD006	EX2102_CTD006_B03_S01	5/20/2021	12:28:41	18.647	23.2109	36.1635	6.8389	228.9377289	24.8362	0.2935	0.2387
CTD006	EX2102_CTD006_B04_S01	5/20/2021	12:28:53	18.569	23.2113	36.1637	6.8174	228.4493284	24.836	0.2691	0.1617
CTD006	EX2102_CTD006_B05_S01	5/20/2021	12:30:08	4.729	23.2132	36.1645	6.8114	227.2283272	24.7757	0.2813	0.1874

CTD006	EX2102_CTD006_B06_S01	5/20/2021	12:30:20	4.302	23.2138	36.1647	6.8188	227.2283272	24.7739	0.2691	0.213
CTD007	EX2102_CTD007_B01_S01	5/21/2021	15:53:03	818.047	9.6172	35.1831	3.8717	207.9365079	30.8676	0.4889	0.0079
CTD007	EX2102_CTD007_B01_S02	5/21/2021	15:53:03	818.047	9.6172	35.1831	3.8717	207.9365079	30.8676	0.4889	0.0079
CTD007	EX2102_CTD007_B02_S01	5/21/2021	15:54:46	806.387	9.6111	35.1828	3.8576	208.4249084	30.816	0.4156	-0.0434
CTD007	EX2102_CTD007_B03_S01	5/21/2021	15:56:09	793.957	9.5914	35.1808	3.8791	208.6691087	30.762	0.379	-0.0434
CTD007	EX2102_CTD007_B04_S01	5/21/2021	16:00:32	649.13	15.7412	36.0762	4.3344	206.2271062	29.5095	0.2813	-0.069
CTD007	EX2102_CTD007_B05_S01	5/21/2021	16:04:54	501.13	18.2169	36.5107	5.7256	204.5177045	28.5781	0.2691	-0.0947
CTD007	EX2102_CTD007_B06_S01	5/21/2021	16:08:17	450.103	18.4933	36.5389	5.4338	204.5177045	28.3048	0.2569	-0.0947
CTD007	EX2102_CTD007_B06_S02	5/21/2021	16:08:17	450.103	18.4933	36.5389	5.4338	204.5177045	28.3048	0.2569	-0.0947
CTD007	EX2102_CTD007_B07_S01	5/21/2021	16:11:22	400.743	19.0396	36.6246	5.9865	204.5177045	28.0115	0.2691	-0.069
CTD007	EX2102_CTD007_B09_S01	5/21/2021	16:19:30	200.508	22.0023	36.8719	6.1087	204.029304	26.5139	0.2691	-0.0434
CTD007	EX2102_CTD007_B10_S01	5/21/2021	16:23:05	150.769	23.4388	36.8809	6.2327	203.2967033	25.8874	0.2691	0.0079
CTD007	EX2102_CTD007_B11_S01	5/21/2021	16:26:11	88.962	25.7141	36.5696	5.7999	202.5641026	24.6922	0.2813	1.2387
CTD007	EX2102_CTD007_B12_S01	5/21/2021	16:29:53	3.965	26.7808	36.3239	6.3179	200.6105006	23.8027	0.2203	-0.2229
CTD008	EX2102_CTD008_B01_S01	5/22/2021	14:00:50	812.341	9.692	35.2147	4.0498	224.0537241	30.8527	0.2691	-0.0434
CTD008	EX2102_CTD008_B01_S02	5/22/2021	14:00:50	812.341	9.692	35.2147	4.0498	224.0537241	30.8527	0.2691	-0.0434
CTD008	EX2102_CTD008_B02_S01	5/22/2021	14:02:06	804.496	9.6888	35.2074	4.0097	224.2979243	30.8123	0.2935	-0.069
CTD008	EX2102_CTD008_B03_S01	5/22/2021	14:03:28	792.416	9.8911	35.1976	3.8106	224.5421245	30.7128	0.2813	-0.0177
CTD008	EX2102_CTD008_B04_S01	5/22/2021	14:07:09	701.474	12.8572	35.6102	3.8765	223.5653236	30.0335	0.2935	-0.069
CTD008	EX2102_CTD008_B05_S01	5/22/2021	14:11:35	551.219	16.5263	36.2175	4.6633	221.6117216	28.9976	0.2813	-0.0434
CTD008	EX2102_CTD008_B06_S01	5/22/2021	14:15:14	450.415	18.0274	36.4552	4.9946	220.3907204	28.3621	0.2935	-0.0434
CTD008	EX2102_CTD008_B06_S02	5/22/2021	14:15:14	450.415	18.0274	36.4552	4.9946	220.3907204	28.3621	0.2935	-0.0434
CTD008	EX2102_CTD008_B07_S01	5/22/2021	14:17:46	400.303	18.83	36.6046	6.103	219.4139194	28.0493	0.2569	-0.0947
CTD008	EX2102_CTD008_B09_S01	5/22/2021	14:25:29	200.067	22.3521	36.8234	6.5262	217.4603175	26.375	0.2691	0.2899
CTD008	EX2102_CTD008_B10_S01	5/22/2021	14:28:15	151.12	23.8775	36.881	6.4049	216.2393162	25.7584	0.2813	0.6233
CTD008	EX2102_CTD008_B11_S01	5/22/2021	14:31:27	93.975	25.3797	36.6726	5.7551	215.2625153	24.8957	0.2935	1.6489
CTD008	EX2102_CTD008_B12_S01	5/22/2021	14:36:32	3.368	26.5167	36.3554	6.4204	212.5763126	23.9079	0.2813	-0.0947

CTD009	EX2102_CTD009_B01_S01	5/23/2021	16:19:44	858.207	6.5322	35.1083	6.1789	201.5873016	31.5122	0.2691	-0.0434
CTD009	EX2102_CTD009_B01_S02	5/23/2021	16:19:44	858.207	6.5322	35.1083	6.1789	201.5873016	31.5122	0.2691	-0.0434
CTD009	EX2102_CTD009_B02_S01	5/23/2021	16:21:11	849.525	6.5965	35.1091	6.1213	201.8315018	31.4633	0.2691	-0.069
CTD009	EX2102_CTD009_B03_S01	5/23/2021	16:22:20	839.668	6.8754	35.1145	5.9174	202.0757021	31.3792	0.2691	-0.0434
CTD009	EX2102_CTD009_B04_S01	5/23/2021	16:26:56	699.881	9.7994	35.2772	4.4262	202.0757021	30.3751	0.2813	-0.069
CTD009	EX2102_CTD009_B05_S01	5/23/2021	16:30:27	600.709	12.5085	35.6239	4.5602	201.3431013	29.6685	0.2691	-0.069
CTD009	EX2102_CTD009_B06_S01	5/23/2021	16:33:40	499.803	15.0173	36.007	5.119	200.3663004	28.9663	0.2691	-0.069
CTD009	EX2102_CTD009_B06_S02	5/23/2021	16:33:40	499.803	15.0173	36.007	5.119	200.3663004	28.9663	0.2691	-0.069
CTD009	EX2102_CTD009_B07_S01	5/23/2021	16:37:34	401.297	17.2739	36.3745	5.5533	199.6336996	28.2745	0.2569	-0.0947
CTD009	EX2102_CTD009_B09_S01	5/23/2021	16:44:44	200.246	21.0485	36.7957	6.3849	198.4126984	26.7232	0.2691	0.4951
CTD009	EX2102_CTD009_B10_S01	5/23/2021	16:47:32	150.534	23.2693	36.9108	5.5066	198.1684982	25.9591	0.2813	0.0848
CTD009	EX2102_CTD009_B11_S01	5/23/2021	16:50:05	102.399	24.5862	36.7857	5.8383	197.6800977	25.2614	0.2813	0.931
CTD009	EX2102_CTD009_B12_S01	5/23/2021	16:54:30	4.071	26.9743	36.3998	6.4009	195.970696	23.7984	0.2935	-0.069
CTD010	EX2102_CTD010_B01_S01	5/24/2021	16:29:25	834.991	6.3495	35.1038	6.3378	192.0634921	31.4305	0.2691	-0.069
CTD010	EX2102_CTD010_B01_S02	5/24/2021	16:29:25	834.991	6.3495	35.1038	6.3378	192.0634921	31.4305	0.2691	-0.069
CTD010	EX2102_CTD010_B02_S01	5/24/2021	16:31:06	825.265	6.9347	35.1175	5.8777	192.5518926	31.3066	0.2813	-0.0434
CTD010	EX2102_CTD010_B03_S01	5/24/2021	16:32:26	815.764	7.3839	35.1268	5.4938	192.5518926	31.1991	0.2935	-0.0434
CTD010	EX2102_CTD010_B04_S01	5/24/2021	16:36:55	700.815	10.4101	35.3469	4.3922	192.3076923	30.3196	0.2935	-0.069
CTD010	EX2102_CTD010_B05_S01	5/24/2021	16:40:57	600.223	13.1623	35.7169	4.7591	191.5750916	29.6002	0.3058	-0.069
CTD010	EX2102_CTD010_B06_S01	5/24/2021	16:44:52	500.282	15.7292	36.1313	5.165	190.8424908	28.8982	0.2813	-0.0434
CTD010	EX2102_CTD010_B06_S02	5/24/2021	16:44:52	500.282	15.7292	36.1313	5.165	190.8424908	28.8982	0.2813	-0.0434
CTD010	EX2102_CTD010_B07_S01	5/24/2021	16:48:46	400.996	17.9798	36.487	5.8058	190.1098901	28.1818	0.2813	-0.0947
CTD010	EX2102_CTD010_B08_S01	5/24/2021	16:52:24	300.074	19.1379	36.6414	6.0556	190.1098901	27.558	0.2813	-0.0947
CTD010	EX2102_CTD010_B09_S01	5/24/2021	16:56:33	200.463	21.0776	36.7966	6.4078	190.1098901	26.7167	0.2935	0.4694
CTD010	EX2102_CTD010_B10_S01	5/24/2021	17:00:00	150.409	23.2994	36.9107	5.1832	190.8424908	25.9496	0.2813	0.3156
CTD010	EX2102_CTD010_B11_S01	5/24/2021	17:02:24	108.687	25.4561	36.7864	5.6028	190.1098901	25.0216	0.2935	1.0848
CTD010	EX2102_CTD010_B12_S01	5/24/2021	17:07:26	3.925	27.1257	36.3708	6.3898	188.1562882	23.7273	0.3302	-0.1203

CTD011	EX2102_CTD011_B01_S01	5/25/2021	13:52:16	1057.58	4.4534	35.0056	7.949	194.2612943	32.6456	0.2813	-0.069
CTD011	EX2102_CTD011_B01_S02	5/25/2021	13:52:16	1057.58	4.4534	35.0056	7.949	194.2612943	32.6456	0.2813	-0.069
CTD011	EX2102_CTD011_B02_S01	5/25/2021	13:56:26	899.323	4.8939	35.0363	7.663	194.993895	31.8822	0.2935	-0.069
CTD011	EX2102_CTD011_B03_S01	5/25/2021	13:59:23	799.899	10.6517	35.3706	4.3958	194.2612943	30.7366	0.2935	0.0079
CTD011	EX2102_CTD011_B04_S01	5/25/2021	14:02:58	700.729	13.2956	35.74	4.7385	193.040293	30.0365	0.2813	-0.069
CTD011	EX2102_CTD011_B05_S01	5/25/2021	14:06:29	600.893	15.1973	36.0348	5.1827	192.5518926	29.3933	0.2813	-0.069
CTD011	EX2102_CTD011_B06_S01	5/25/2021	14:10:21	499.764	17.4792	36.4039	5.6273	192.0634921	28.6785	0.2569	-0.0947
CTD011	EX2102_CTD011_B06_S02	5/25/2021	14:10:21	499.764	17.4792	36.4039	5.6273	192.0634921	28.6785	0.2569	-0.0947
CTD011	EX2102_CTD011_B07_S01	5/25/2021	14:15:39	401.521	18.6573	36.5856	6.0839	192.3076923	28.0854	0.2691	-0.0947
CTD011	EX2102_CTD011_B08_S01	5/25/2021	14:22:34	300.84	19.4806	36.6858	6.2731	193.5286935	27.5047	0.2691	-0.0947
CTD011	EX2102_CTD011_B10_S01	5/25/2021	14:31:48	201.092	20.8059	36.7827	6.385	194.993895	26.7841	0.2813	0.3669
CTD011	EX2102_CTD011_B11_S01	5/25/2021	14:36:03	96.723	25.6753	36.7481	5.7321	194.017094	24.8727	0.2935	1.0335
CTD011	EX2102_CTD011_B12_S01	5/25/2021	14:39:49	3.27	27.1982	36.3508	6.4	192.3076923	23.686	0.2569	-0.069
CTD012	EX2102_CTD012_B01_S01	5/26/2021	14:24:27	70.906	22.4977	36.2071	6.7005	197.4358974	25.3033	0.6421	1.8797
CTD012	EX2102_CTD012_B02_S01	5/26/2021	14:24:36	71.608	22.4101	36.203	6.6868	197.4358974	25.3283	0.6164	1.8797
CTD012	EX2102_CTD012_B03_S01	5/26/2021	14:26:13	30.77	25.4258	36.3713	6.5271	195.970696	24.3804	0.5908	0.1105
CTD012	EX2102_CTD012_B04_S01	5/26/2021	14:26:24	31.041	25.4233	36.3719	6.5868	195.970696	24.3828	0.6164	0.0592
CTD012	EX2102_CTD012_B05_S01	5/26/2021	14:27:37	2.466	26.0392	36.3961	6.5104	195.4822955	24.0855	0.5652	-0.069
CTD012	EX2102_CTD012_B06_S01	5/26/2021	14:27:47	3.286	26.0468	36.3979	6.4906	195.2380952	24.0879	0.5652	0.1361

7.3 Acoustic Operations Results

During EX-21-02, multibeam mapping operations results included 3,345 linear km mapped and 9,669 km^2 covered, predominantly in previously unmapped areas and all in U.S. waters. **Figure 12** shows previously compiled publically available bathymetry in gray and the EX-21-02 EM 304 bathymetry in color.

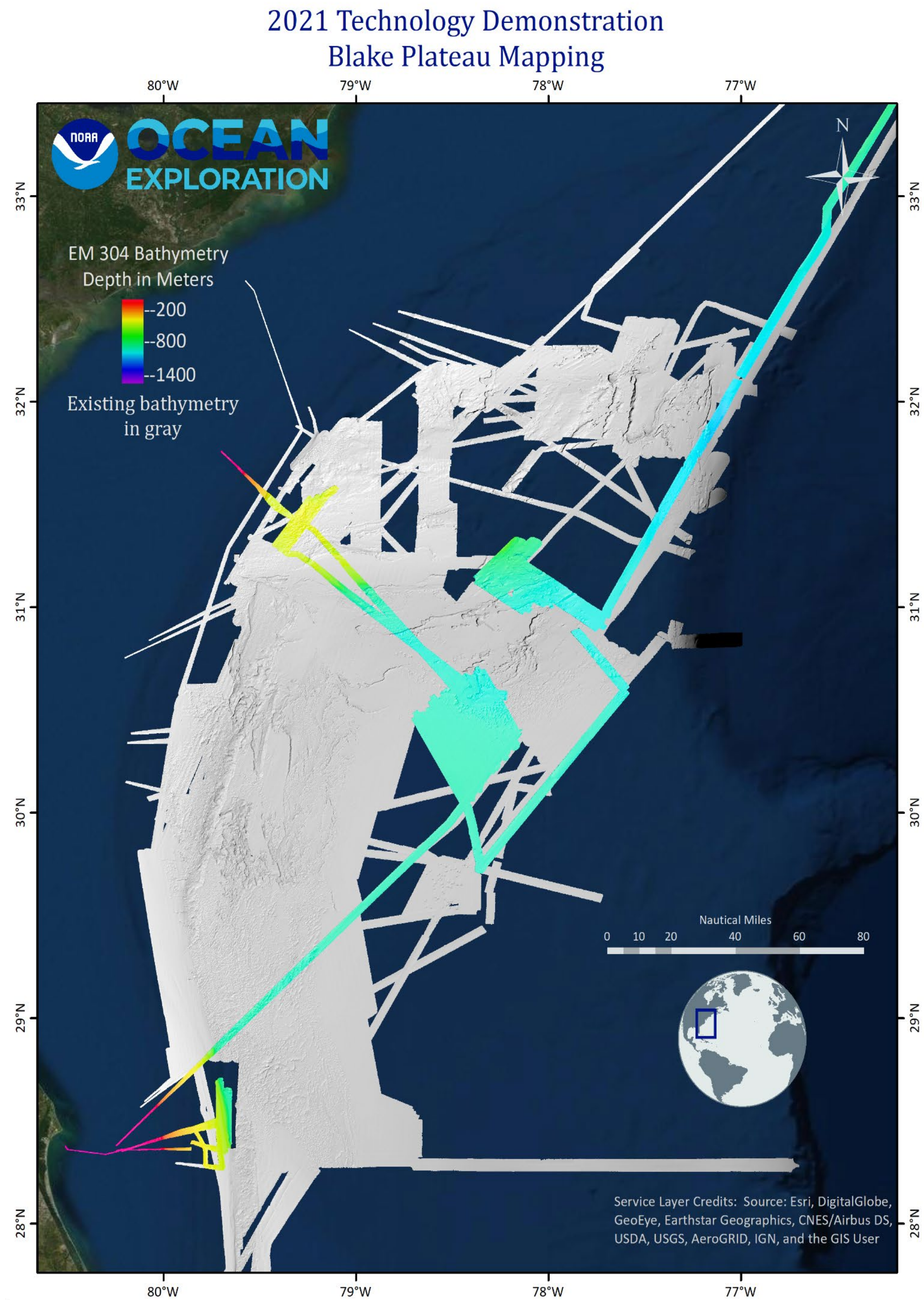

Figure 12. EX-21-02 EM 304 bathymetry (color) shown along with previously compiled publically available bathymetry (grey).

In addition to the focused mapping completed on the Blake Plateau filling in gaps in unmapped areas, several focused surveys were done in partnership with the Monitor National Marine Sanctuary (MNMS). **Figures 13 – 15** display some of the targets that were mapped.

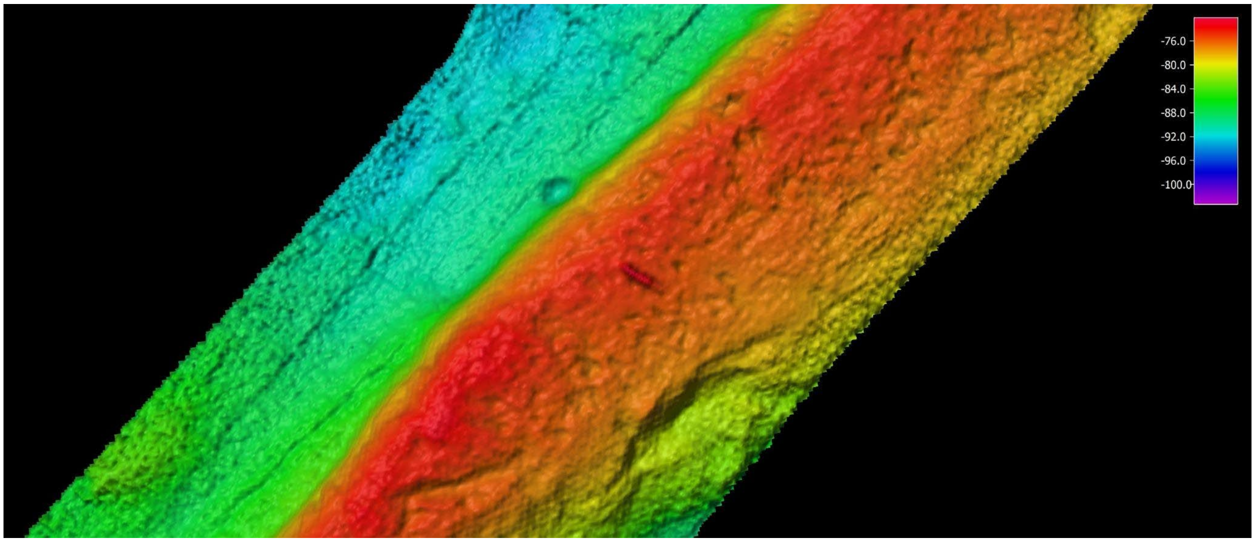

Figure 13. Target CH00013 in EM 304 bathymetry.

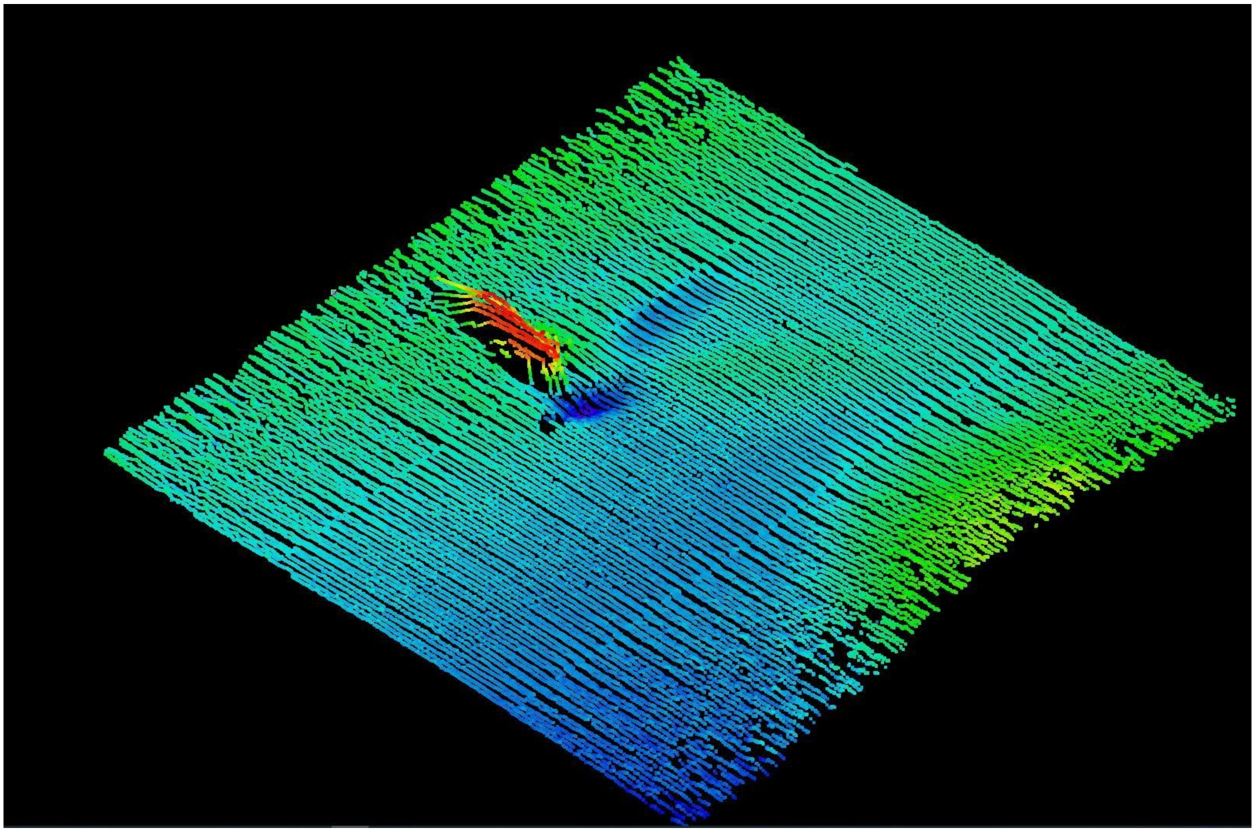

Figure 14. U.S.S. *New Jersey*, a WWI-era battleship purposely sunk for military aviation training (personal correspondence W. Sassorossi, Research Coordinator, MNMS, June 2021). Oblique view of EM 304 multibeam sonar point cloud.

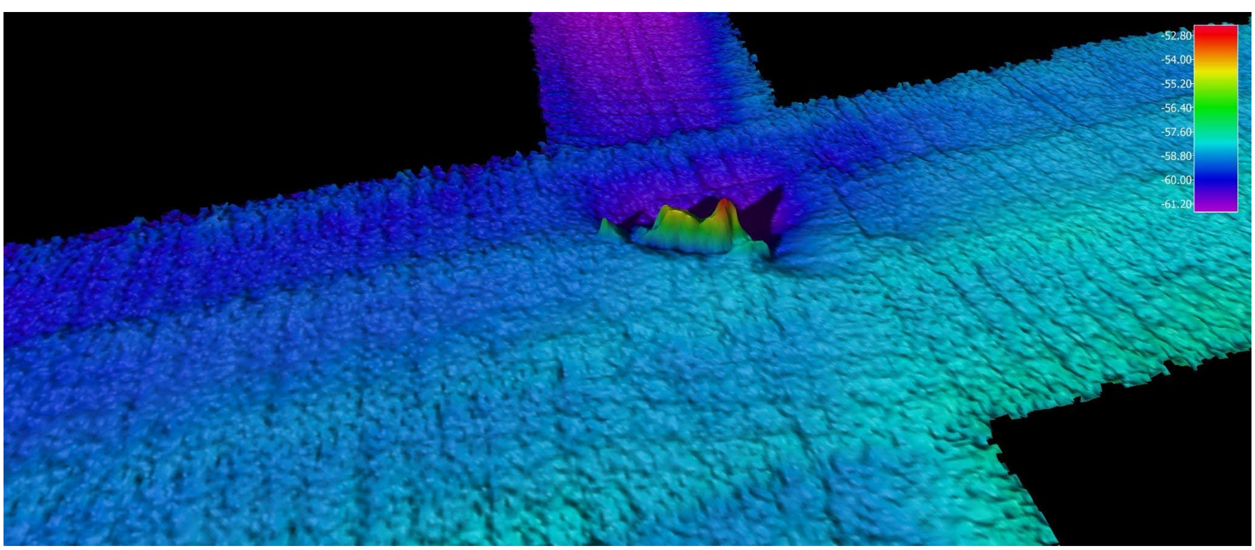

Figure 15. *Papoose* wreck in two-meter resolution EM 304 3D bathymetry grid.

Certain acoustic data were collected per request from researchers at the Center for Coastal and Ocean Mapping/Joint Hydrographic Center, University of New Hampshire (CCOM/JCH). Further information about the mapping conducted during EX-21-02, including data quality assessments, is in the EX-21-02 mapping data report, Mapping Data Acquisition and Processing Report: EX-21-02, 2021 Technology Demonstration (AUV and Mapping)[17] (Sowers et al., 2021).

7.3.1 Acoustic Operations Data Access

Multibeam Sonar (Kongsberg EM 304)

The multibeam dataset for the expedition is archived at NCEI and accessible through their Bathymetric Data Viewer. To access these data, click on the Search Bathymetric Surveys button, select "NOAA Ship *Okeanos Explorer*" from the Platform Name dropdown menu, and "EX2102" from the Survey ID dropdown menu. Click OK, and the ship track will appear on the map. Click the ship track for options to download data.

Sub-Bottom Profiler (Knudsen Chirp 3260)

The sub-bottom was operated during multibeam mapping operations. These data are archived at NCEI and accessible through the Trackline Geophysical Data Viewer. To access these data, select "Subbottom Profile" under Marine Surveys and click on Search Marine Surveys. In the pop-up window, select "EX2102" in the Filter by Survey IDs dropdown menu. Click OK, and the ship track will appear on the map. Click the ship track for options to download data. For any issues contact ncei.info@noaa.gov.

Split-Beam Sonars (Simrad EK60 and EK80)

EK60 and EK80 water column data for EX-21-02 are archived at NCEI and available through the Water Column Sonar Data Viewer. To access these data, click on the Additional Filters button, deselect "All" next to Survey ID, and select "EX2102" from the Survey ID list. Click OK, and the ship track will appear on the map. Click on the ship track for options to download data.

Acoustic Doppler Current Profilers (Teledyne Marine Workhorse Mariner and Teledyne Ocean Surveyor ADCPs)

Please contact ncei.info@noaa.gov for information on the ADCP data collected onboard NOAA Ship *Okeanos Explorer*.

[17] doi:10.25923/edtg-dq62

7.4 Conductivity, Temperature, and Depth Measurements

CTD profile data and CTD Summary forms from EX-21-02 are archived at NCEI and available through NOAA Ocean Exploration's Digital Atlas. To access these data, click on the Search tab, enter "EX2102" in the Enter Search Text field, and click Search. Click on the point that represents EX-21-02 to access data options. In the pop-up window, select the Data Access tab for a link to download the CTD profile data.

7.5 Sun Photometer Measurements

Sun photometer measurements are available through NASA's Marine Aerosol Network. Access these data by searching the table for "2021," "*Okeanos Explorer*," and "U.S. Southeast." Click on the links to download the data. (Note: There may be more than one entry for *Okeanos Explorer* in a region in a given year.)

7.6 Engagement

Mission personnel approached the virtual environment of a world still adapting to the COVID-19 pandemic as both a challenge and opportunity. Public engagement efforts during EX-21-02 took full advantage of the additional reach afforded by a world that has shifted towards virtual interactions as the norm. These activities leveraged NOAA Ocean Exploration's extensive experience utilizing telepresence to engage with virtual audiences. These efforts allowed for direct engagement with audiences around the world and created space for inquiry and dialogue between experts and the public. Highlights are listed below:

- On Wednesday, May 5 at 7 p.m. ET, a live event introducing the 2021 Technology Demonstration was hosted by NOAA Ocean Exploration's Rachel Gulbraa. The event featured mission personnel including Expedition Coordinator Mike White, Meredith Everett of the NOAA Northwest Fisheries Science Center, Russell Smith of the NASA Jet Propulsion Laboratory, and Tim Shank of the Woods Hole Oceanographic Institution. The group discussed the technologies to be tested on the expedition and the potential of these technologies for improving our collective understanding of both the ocean here on Earth, and possibly even oceans on other planets. The event was streamed live on Facebook, Twitter, and Youtube, and reached an astounding 169,000 viewers, greatly increasing the typical reach of the office. Reaction highlights included a retweet by the minority side of the Senate Committee on Commerce, Science, and Transportation, who retweeted the event with a specific reference to pending ocean exploration Senate reauthorization legislation.

- On May 13 at 7 pm ET, expedition mission personnel Mike White and Casey Machado (Woods Hole Oceanographic Institution) joined Cecilia D'Anastasio, a reporter with WIRED Games, for a live event to explore the ocean on Planet 4546B — a fictional planet that is the setting of survival adventure video game Subnautica: Below Zero. In the game, players use submersible technology to explore the depths of an underwater world. During the live-streamed event, Cecilia played the single-person video game as Mike and Casey watched after hours from NOAA Ship *Okeanos Explorer* while it was docked in Florida. Mike and Casey were able to comment on how activities in the game are like, or not like, real-life ocean exploration and about the 2021 Technology Demonstration, the tools and technologies being tested during the expedition, partnerships between NOAA, Woods Hole, NASA, and the Ocean Exploration Cooperative Institute, and the value and importance of ocean exploration. This was an exciting opportunity for NOAA Ocean Exploration to reach a new and different audience, diving into the world of live streaming and online gamers.

- Live video feeds received nearly 20,000 views during the expedition, with nearly 5,000 visits to expedition-specific web content.

- 22 news/web articles covered EX-21-02. Stories appeared in national and local media outlets and on websites throughout the country, including Forbes, Wired, Space.com, NASA, Syfy Wire, Hydro International and SciTechDaily. This wide diversity of publication type amplified the impact of the expedition by bringing coverage to new audiences.

The following expedition features were composed during the expedition and are available from NOAA Ocean Exploration’s website (oceanexplorer.noaa.gov)

- Terrain Relative Navigation: From Mars to the Deep Sea
- Ocean Worlds
- The Hadal Zone: *Aqua Incognita*
- Orpheus Class Vehicles
- Testing the Use of Environmental DNA to Explore the Deep Ocean
- Introducing the NOAA Ocean Exploration Cooperative Institute
- Live Interaction Dives Into a Fictional Ocean to Learn About A Real One (Ours)
- 2021 Technology Demonstration: Expedition Plan

8. Summary

EX-21-02 was the first Ocean Exploration Cooperative Institute supported project on NOAA Ship *Okeanos Explorer*. The expedition demonstrated successful integration of *Orpheus* AUV operations during NOAA Ocean Exploration expeditions on NOAA Ship *Okeanos Explorer*. EX-21-02 was the third NOAA Ocean Exploration expedition on NOAA Ship *Okeanos Explorer* using AUVs and the first with multiple AUVs. By the end of the expedition, the team was able to complete regular AUV deployments in support of exploration priorities such as ground-truthing mapping data and identifying deep-sea coral and sponge habitats. These AUVs have the potential for future use on NOAA Ocean Exploration expeditions, especially as they have the ability to reach hadal depths, a capability that the office does not currently have. Because of the AUV's relatively small operations footprint, a swarm approach with multiple vehicles is also possible. The Orpheus class AUVs have the potential to support both seafloor and water column exploration.

EX-21-02 also successfully piloted eDNA operations, including onboard filtering and storage of samples, robust metadata documentation, and development of standard operating procedures to support eDNA water sample processing. eDNA data are important for exploration and characterization purposes, and as a baseline deep-sea observation supporting several high priority exploration variables. eDNA operations support NOAA priorities as identified in the NOAA 'Omics Strategic Plan and the NOMEC Implementation Plan.

The following section describes the key accomplishments achieved during the EX-21-02 2021 Technology Demonstration expedition.

Data Collection:

- Acquired 724 GB of down-looking 4K video footage.
- Surveyed 30 linear kilometers of seafloor with the AUVs (16.40 hours of bottom time) and completed 33 hours of in-water AUV operations.
- Completed AUV surveys and video footage of Blake Plateau coral mound features and associated habitats and biota.
- Performed the first autonomous in-situ electrochemical sensing via an ISEA-X instrument (Analytical Instrument Systems). Obtained 1,624 chemical scans from near bottom and water column, and sent data from the instrument to a shore lab in less than 20 minutes following dives.
- Completed 12 CTD rosette casts collected over 120 eDNA samples (plus control samples) over a variety of seafloor features and in different water masses.

- Pioneered and developed standard operating procedures for the collection, processing, and storage of water column eDNA samples onboard NOAA Ship *Okeanos Explorer*.
- Completed multibeam sonar mapping operations of 9,669 km^2 of seafloor, all in U.S. waters.

Advancements in Orpheu*s* class AUV Technology:

- Completed the inaugural dive of AUV *Eurydice.*
- Completed 7 dives with AUV *Orpheus* from 12 to 866 meters. *Orpheus* only had 3 dives prior to EX-21-02.
- Collected AUV odometry data and visual data to advance the development of Terrain Relative Navigation (TRN).
- Demonstrated Orpheus capability of moving at high speeds over the seafloor - averaging 2:04 hours of bottom time per dive covering ~4km, using approximately 50% thrust.

Advancement in Ocean Exploration Concepts of Operation:

- Developed a synergistic concept of operations for efficient concurrent Orpheus-class AUV, CTD/eDNA, and mapping operations. First exploration mapping was conducted using the multibeam sonar to target features (in this case cold-water coral mounds) for further AUV investigation. Next, water column current velocities and directions were assessed using ADCPs to locate an optimal AUV drop position that would enable the AUV to largely drift toward the desired seafloor feature of interest as it descended. The ship then deployed the AUV at the drop point and slowly moved toward the bottom target to maintain positional tracking of the AUV with the USBL. Ship CTD rosette casts with eDNA water sample collection were then completed over the feature being explored by the AUV. It is expected that the eDNA samples will be useful for both validating biological communities imaged by the AUV as well as detecting the presence of species not represented in the imagery. This concept of operations became highly efficient during the expedition and demonstrated the ability to maximize the synergies of these capabilities for science.

9. References

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Appendix A: NEPA Categorical Exclusion

Categorical Exclusion (CE) Evaluation Worksheet

Project Identifier: EX2102

Date Review Completed: 3/25/2021

Completed by: Amanda Maxon, Environmental Compliance Specialist, Contractor

OAR Functional Area: OER

Worksheet File Name: 2021-03-OER-E3-EX2102

Step 1. CE applicability

1. **Is this federal financial assistance, including via grants, cooperative agreements, loans, loan guarantees, interest subsidies, insurance, food commodities, direct appropriations, and transfers of property in place of money?**

   yes

2. **What is the proposed federal action?**
   The proposed action is to test out two autonomous underwater vehicles (AUV) onboard NOAA Ship Okeanos Explorer. The AUVs are in development by Woods Hole Oceanographic Institute, with support for field operations from NOAA/OAR/OER. The AUV operations will include sending the vehicles down to depths between 20-4000m to take images of the AUV in operation and to calibrate equipment systems for field operations. Support from the ship will include baseline mapping using the ship's EM304 multibeam system, EK60/EK80 split-beam water column sonar, Knudson Chirp 3260 sub-bottom profiler, acoustic doppler current profiler, and CTD casts. During CTD casts water column samples will also be taken using the 10L Niskin bottles on the CTD rosette. Approximately 120 water samples will be collected. Other additional operations include use of a small boat to calibrate TRN software using underwater acoustics and taking underwater images of the AUV. The expedition is currently scheduled to start in Port Canaveral, Florida on May 14, 2021 and end in Norfolk, Virginia on May 27, 2021. The exact start and end dates may vary by a few days or weeks depending on weather and other logistical considerations.

   Operations will focus off of the East Coast of the United States, in largely unexplored (>200m) of water. This action demonstrates independent utility and is not connected to

1

any other federal action.

3. **Which class of CE in Appendix E of the NAO 216-6A Companion Manual is applicable to this action and why?**
   a. E3: Activities to collect aquatic, terrestrial, and atmospheric data in a non-destructive manner.
   b. The scope of this action is consistent with Categorical Exclusion E3 in Appendix E of the Companion Manual to NOAA Administrative Order (NAO) 216-6A.
      - Use of mobile platforms (e.g., ships, aircraft, balloons, vehicles) to study biological, chemical, or physical processes.
      - Use of conductivity, temperature, and depth instruments or a moving vessel profiler from a platform, including the use of drop cameras.
      - Deployment, operation, and retrieval of a limited number of ROVs, ASVs, AUVs, buoys, moorings, or similar instrumentation to conduct non-destructive sampling and collection of data from those instruments once installed, including physical, chemical, and biological measurements, and visual data.

Step 2. Extraordinary Circumstances Consideration

4. **Would the action result in adverse effects on human health or safety that are not negligible?**

   No, the actions of the NOAA Ship Okeanos Explorer operating in remote deep-sea (>200m) areas located offshore the United States East coast starting from Port Canaveral, Florida to Norfolk, Virginia. All operations are underwater and therefore have no human presence. This action does not involve any procedures or outcomes known to result in impacts on human health and safety.

5. **Would the action result in adverse effects on an area with unique environmental characteristics that are not negligible?**

   This expedition may include operations within Stetson-Miami Terrace Deepwater Coral HAPC. OER is working with regional management groups to ensure that impacts will be negligible, and that operations will address the management and science needs of the special area, as well as those of the broader region. OER will use input from regional management authorities that are familiar with these areas in order to ensure no more than negligible effects on any areas with potentially unique environmental characteristics.

6. **Would the action result in adverse effects on species or habitats protected by the ESA, MMPA, MSA, NMSA, or MBTA that are not negligible?**

OER has taken measures to ensure that any effects on species or habitats protected by the ESA, MMPA, MSA or NMSA meet the definition of negligible. In 2018, an informal consultation was initiated under Section 7 of the Endangered Species Act (ESA), requesting NOAA Fisheries' Protected Resources Division concurrence with our Biological Evaluation determining that NOAA Ship Okeanos Explorer operations conducted during the 2018-2019 field seasons are not likely to adversely affect ESA-listed marine species. The informal consultation was completed on August 8, 2018 when OER received a signed letter from the Chief ESA Interagency Cooperation Division in the NOAA Office of Protected Species, stating that NMFS concurs with OER's determination that operations conducted during NOAA Ship Okeanos Explorer 2018-2019 field seasons are not likely to adversely affect ESA-listed marine species. A Re-initiation of ESA Section 7 Letter of Concurrence was completed for the FY20 cruise season. ESA Section 7 Letter of Concurrence was received for the Okeanos Explorer's FY21 field season on February 4, 2021 which incorporated the usage of new technologies and regions of interest. Which will be provided as an appendix in the 2021 Project Instructions.

Given the offshore focus of most of our proposed work, it is improbable that we will encounter marine mammals protected under the MMPA, or sea birds protected under the MBTA. If we did encounter any such protected animals, our impacts would be negligible because of the best management practices to which we adhere to avoid or minimize environmental impacts. These best management practices are all outlined in the appendices of the in the EX-21-02 project instructions. OER also initiated a request for an abbreviated Essential Fish Habitat (EFH) consultation for expeditions by NOAA Ship Okeanos Explorer in 2021 to the Greater Atlantic Region. In which OER received a Letter of Acknowledgement from the Assistant Regional Administrator for the NOAA Office of Habitat Conservation on March 10, 2021, stating that these expeditions will not adversely impact EFH. This letter will be provided in appendices of the EX FY21 Project Instructions.

Permits and compliance for each expedition will be included in the appendices of the project instructions and final report.

7. Would the action result in the potential to generate, use, store, transport, or dispose of hazardous or toxic substances, in a manner that may have a significant effect on the environment?

No. The cruise operations will be in the compliance with FEC 07 Hazardous Materials and Hazardous Waste Management Requirements for Visiting Scientific Parties (or the OMAO procedure that supersedes it) to ensure generation, use, storage, transport, and disposal of such substances will not result in significant impacts.

8. Would the action result in adverse effects on properties listed or eligible for listing on the National Register of Historic Places authorized by the National Historic

Preservation Act of 1966, National Historic Landmarks designated by the Secretary of the Interior, or National Monuments designated through the Antiquities Act of 1906; Federally recognized Tribal and Native Alaskan lands, cultural or natural resources, or religious or cultural sites that cannot be resolved through applicable regulatory processes?

The proposed action will not result in adverse effects that cannot be resolved through applicable regulatory processes since we will not be operating within listed or eligible properties, lands, resources or sites coming under the umbrella of protection referenced above.

9. **Would the action result in a disproportionately high and adverse effect on the health or the environment of minority or low-income communities, compared to the impacts on other communities (EO 12898)?**

The NOAA Ship Okeanos Explorer will be operating in the remote and offshore areas along the Southeast part of the United States during EX-21-02. There are no communities within or near the geographic scope of the cruise and the cruise does not involve actions known or likely to result in adverse impacts on human health.

10. **Would the action contribute to the introduction, continued existence, or spread of noxious weeds or nonnative invasive species known to occur in the area or actions that may promote the introduction, growth, or expansion of the range of the species?**

During EX-21-02, NOAA Ship Okeanos Explorer will not make landfall in areas other than commercial ports in Port Canaveral, Florida to Norfolk, Virginia. The ship and OER mission team will comply with all applicable local and federal regulations regarding the prevention or spread of invasive species. At the completion of every ROV dive or CTD cast, the equipment will be thoroughly rinsed with fresh water and completely dried to prevent spreading organisms from one site to another. Also the Engineering Department aboard the NOAA Ship Okeanos Explorer attends yearly Ballast Management Training in accordance with NOAA Form 57-07-13 NPDES VGP Annual Inspection and Report to prevent the introduction of invasive species.

11. **Would the action result in a potential violation of Federal, State, or local law or requirements imposed for protection of the environment?**

The proposed action will not result in a potential violation of Federal, State, or local law or requirements imposed for protection of the environment. The expedition coordinator obtained authorizations for this expedition via several consultations on ESA Section-7 and EFH outlined in sections 4-7 above.

12. Would the action result in highly controversial environmental effects?

No, the exploration activities will be localized and of short duration in any particular area at any given time. Given the project's scope and breath, no notable or lasting changes or highly controversial effects to the environment will result.

13. Does the action have the potential to establish a precedent for future action or an action that represents a decision in principle about future actions with potentially significant environmental effects?

While each cruise contributes to the overarching goal of exploring, mapping, and sampling the ocean, every cruise is independently useful and not connected to subsequent federal actions.

14. Would the action result in environmental effects that are uncertain, unique, or unknown?

The techniques and equipment used are standard for this type of field study, and the effects are well known.

15. Does the action have the potential for significant cumulative impacts when the proposed action is combined with other past, present and reasonably foreseeable future actions, even though the impacts of the proposed action may not be significant by themselves?

By definition, actions that a federal agency classifies as a categorical exclusion have no potential, individually or cumulatively, to significantly affect the environment. This cruise is consistent with a class of CE established by NOAA and there are no extraordinary circumstances for this action that may otherwise result in potentially significant impacts.

CE Determination

☒ I have determined that a Categorical Exclusion is the appropriate level of NEPA analysis for this action and that no extraordinary circumstances exist that would require preparation of an environmental assessment or environmental impact statement.

☐ I have determined that an environmental assessment or environmental impact statement is required for this action.

Signature: SOSSA.GENENE.FISHER.1403930306 Digitally signed by SOSSA.GENENE.FISHER.1403930306 Date: 2021.04.08 16:42:37 -04'00'

Signed by: Genene Fisher, Deputy Director, OER

Date Signed: 4/8/2021

Appendix B: Standard Operating Procedures, Collecting environmental DNA (eDNA) samples with the CTD rosette on NOAA Ship Okeanos Explorer

PROCESS OWNER
NOAA Ocean Exploration

REVISION HISTORY			
REV	Description of Change	Author	Effective Date
0	Initial release	Katharine Egan, Meredith Everett, Kimberly Galvez, Danielle Power, Mike White	May 2021
1			

REFERENCE DOCUMENTS		
EX Sampling Supplies Inventory	OER Internal Products and Services Form	
Everett & Park (2018)		
Longmire et al. (1997)		

Brief description of purpose of this SOP.
Process of collecting and filtering water samples via the CTD rosette for environmental DNA (eDNA) processing.

Table of Contents

During Mobilization

Data management preparation

Explanation: The final products from the expedition involving eDNA and CTD rosette casts are as follows. Be sure to keep all of these data organized in the appropriate Google Drive or shared drive folders.

- CTD data for each cast, both in the .hex file format and the .cnv format.
    - Level_00 files
        - *.hex, *.hdr, *.xmlcon, *.bl
    - Level_01 files
        - *.cnv
            - The *.cnv file is just the downcast processed and QC/QA for erroneous data
- A CTD rosette summary form filled out for each CTD cast conducted
    - CTD Rosette Summary Form Template
        - Form has been reviewed for NOAA Ocean Exploration branding and 508c compliance for FY21.
- A spreadsheet containing summary information about all of the CTD casts conducted on the expedition.

- example: EX2102_CTD_Summary_Table

- A spreadsheet containing metadata for each Niskin bottle fired across the entire expedition.
  - example: EX2102_CTDrosette_WaterSample_Metadata

- The calibration files for the sensors on the CTD rosette. These are typically supplied by the Senior Survey Technician (SST) and one copy can be archived with the data set.

1. Make a copy of the CTD rosette cast summary form template and fill out all information that will not change for the duration of the expedition (e.g. Expedition Coordination, Mapping Lead, Science Leads, location, etc.) to generate an expedition-specific template.
   a. Use cruise folder on Google Drive
2. Create a Google spreadsheet with columns that contain the following information:

Column Name	**Format**	**Description**
Expedition	EX-YY-XX	Expedition name
CTD Cast Name	EXYYXX_CTDXXX_YYYYMMDD	CTD cast name
CTD Number	CTDXXX	CTD number
Date	YYYY-MM-DD (UTC)	Date the cast took place (UTC time)
Deployment Time	HH:MM:SS (UTC)	Time the cast deployment took place (UTC time)
Deployment Latitude	##.##### (decimal degrees)	Latitude of where the cast deployment took place
Deployment Longitude	##.##### (decimal degrees)	Longitude of where the cast deployment took place
Maximum Depth	#### (meters)	
Recovery Time	HH:MM:SS (UTC)	Time of where the cast recovery took place (UTC time)
Recovery Latitude	##.##### (decimal degrees)	Latitude of where the cast recovery took place
Recovery Longitude	##.##### (decimal degrees)	Longitude of where the cast recovery took place
Number of Water Samples	#	Number of water samples collected the CTD rosette case
Number of Blanks	#	Number of blank samples run for the CTD rosette cast

3. Create a blank Google spreadsheet to store the associated Scientific Computer System (SCS) generated data (see next section).

4. On a shared ship drive (typically 'publicdata', 10.10.2.20), create the following folder structure. This is where screenshots and the CTD data will be stored.
    a. publicdata:/EXYY##
        i. CTD_eDNA
            1. CTD_Data
                a. Level_00
                b. Level_01
            2. Screenshots
                a. EK
                b. SeaBird
                c. Other

Setting up an SCS event with the SST

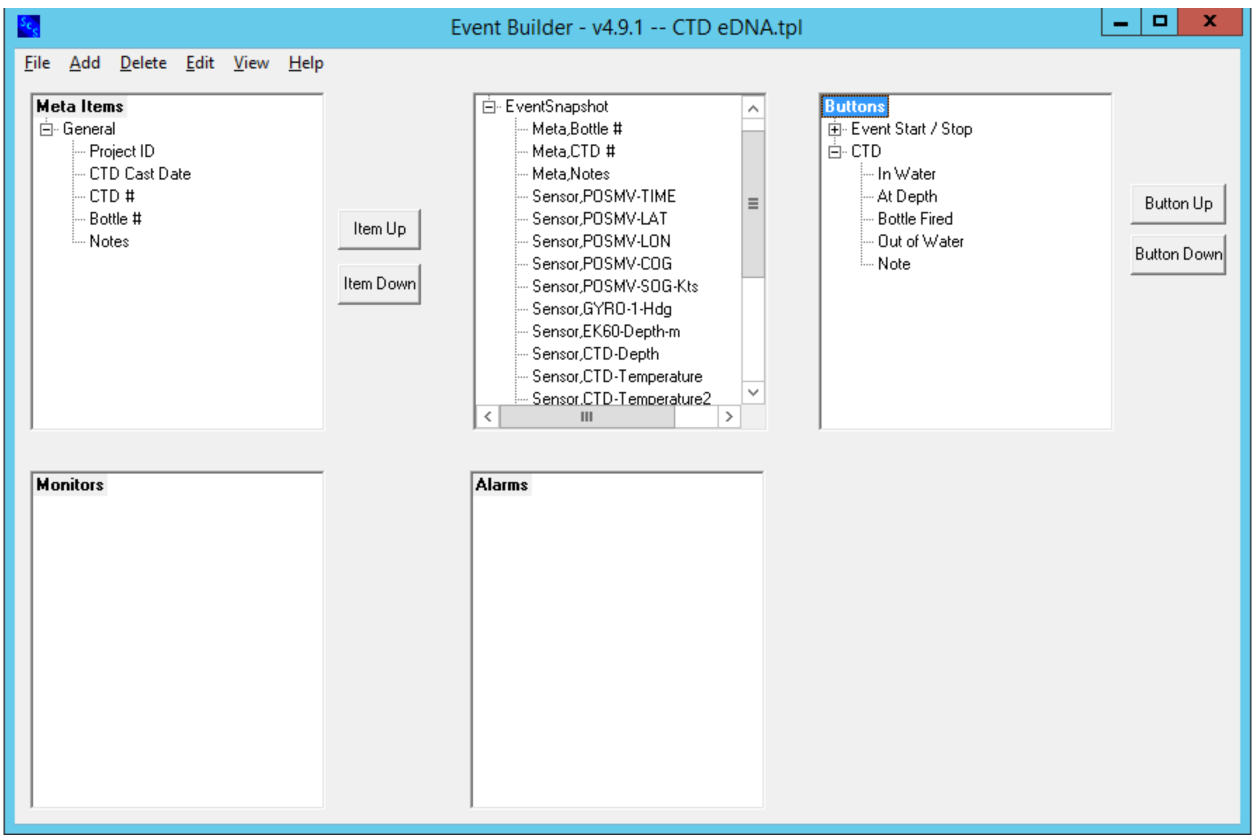
Explanation: This step involves working with the SST to create a SCS event in order to capture the timestamp and associated metadata every time a Niskin bottle is fired on the CTD. The data generated here will go into the aforementioned SCS spreadsheet on Google Drive. A screenshot of the SCS event is below.

1. Work with the SST to set up an SCS event for the CTD rosette data.

2. Ask for the following information for the SCS event:
    a. Button marking the time the CTD is in the water
    b. Button marking the time the CTD is at depth
    c. Button marking the time each Niskin bottle is fired
    d. Button marking the time the CTD is out of the water
    e. Button marking any additional notes

3. Each button in the SCS event should record the data at the time each of these buttons are pressed to an output file. These data should include:
    a. Date
    b. Time (UTC)
    c. Button
    d. Bottle #
    e. CTD #
    f. Notes
    g. POSMV-TIME (UTC)
    h. POSMV-LAT (decimal degrees)
    i. POSMV-LON (decimal degrees)

j. POSMV-COG (degrees)
k. POSMV-SOG (Kts)
l. GYRO-1-Hdg (degrees)
m. EK60-Depth (meters)
n. CTD-Depth (meters)
o. CTD-Temperature (deg C)
p. CTD-Temperature2 (deg C)
q. CTD-Salinity1(PSU)
r. CTD-Salinity2 (PSU)
s. CTD-Oxygen SBE43 [mg/l]
t. CTD-Oxygen Reduction Potential (mV)
u. CTD-Density [kg/m^3]
v. CTD-Fluorescence [mg/m^3]
w. CTD-Turbidity (NTU)
x. CTD-Altimeter (meters)



Preparing the Longmire's buffer solution

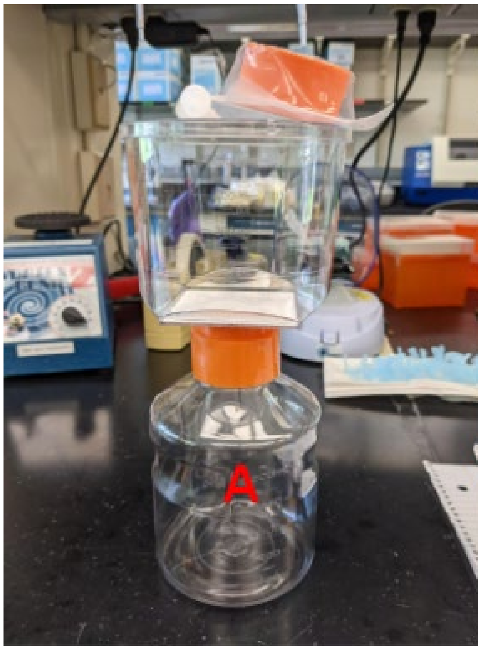
Explanation: These are the supplies needed to make the Longmire's buffer solution, which is a solution used to keep DNA stable at room temperature. The Longmire's buffer solution can be made at any time prior to the start of the expedition during mobilization and it has a shelf life of several years. The filter must be completely submerged in the buffer solution once DNA is captured on the filter. To make 1L of Longmire's buffer:

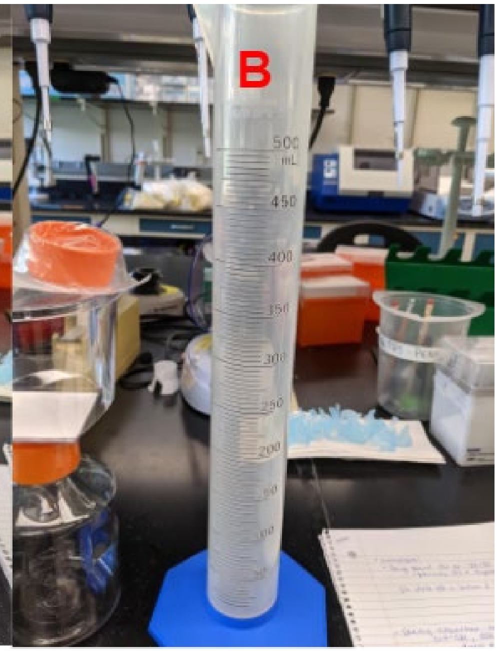
Supply	Quantity	Consumable
1M Tris (pH 8.0)	100 mL	Yes
0.5M EDTA (pH 8.0)	200 mL	Yes
5M NaCl	2 mL	Yes
10% SDS	50 mL	Yes
Autoclaved or Molecular Grade Water	648 mL	Yes
0.22 µm Nalgene filter with bottle attachment	1	Yes
1000 mL clean graduated cylinder	1	No

Explanation: This step involves preparing the Longmire's buffer, which is a solution that allows the DNA on the 0.45 µm filters to be stably stored at room temperature. This recipe makes 1 L of Longmire's buffer. The solution is sterilized using a Nalgene filter unit containing both a built-in 0.22 µm filter and storage bottle. The 0.22 µm filter size will remove microbes and other contaminants from the solution. The buffer solution should be created at the start of the field season and can be used throughout the field season. It is stable at room temperature for many years.

1. Put on gloves.

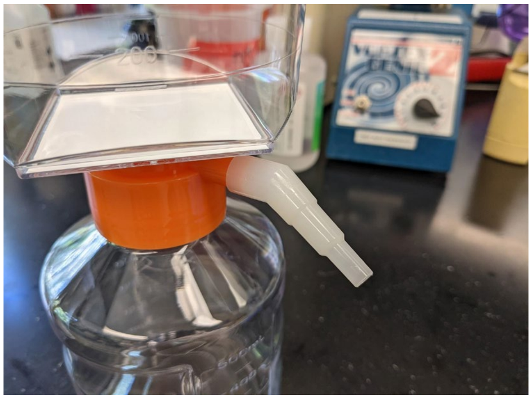
2. Clean the container, bench, and gloves with either DNA Away or 5-10% bleach solution. The container can be a 1L graduated cylinder or sterilized 1-2L container such as a Nalgene bottle. If sterilizing the container with 10% bleach, make sure it is rinsed very well before using.
    a. Sterile Vacuum Bottle (A) and sterile graduated cylinder (B) for making Longmire's buffer.



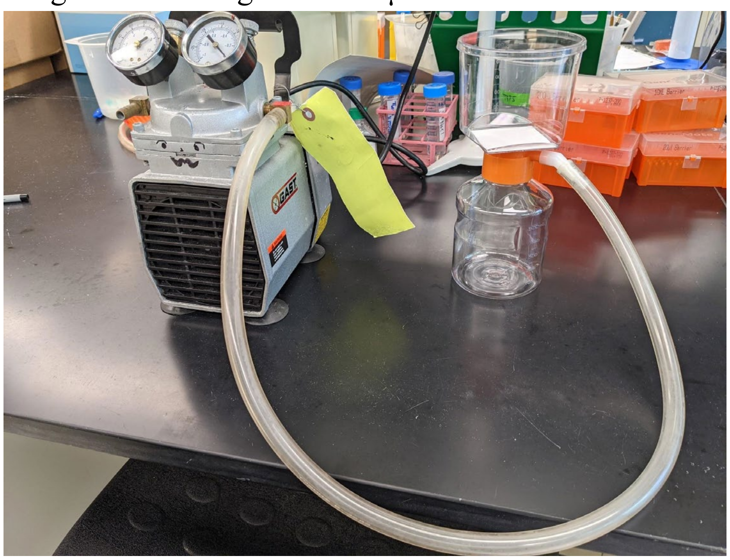


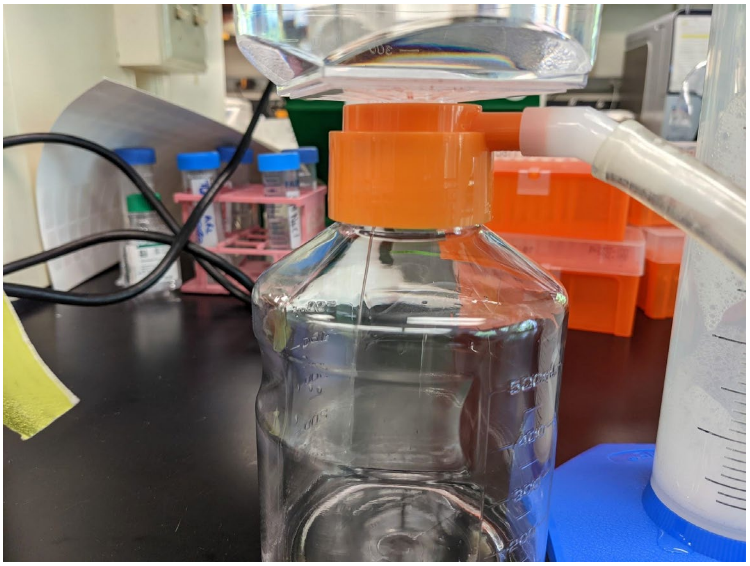
3. Measure and combine the 100 mL 1M Tris, 200 mL 0.5M EDTA, 2 mL 5M NaCl, 50 mL 10% SDS, and 648 mL of molecular grade water together into the sterilized container (1 L graduated cylinder or 1-2 L Nalgene).

4. Make sure the vacuum port tube (provided with filter assembly) on the Nalgene 0.22 µm filter assembly is installed and connected to the vacuum (picture below).

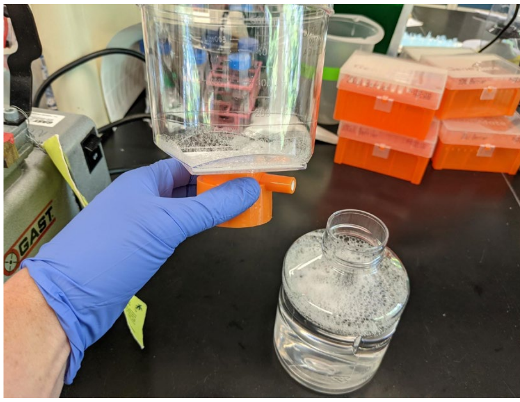


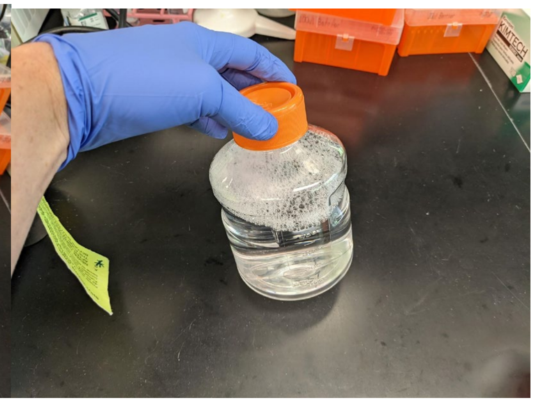
5. Remove the lid from the top chamber and pour the mixed solution into the top chamber of the Nalgene 0.22 µm filter assembly. Apply vacuum, drawing the solution into the lower storage bottle through the 0.22 µm filter to sterilize the mixture (pictures below).

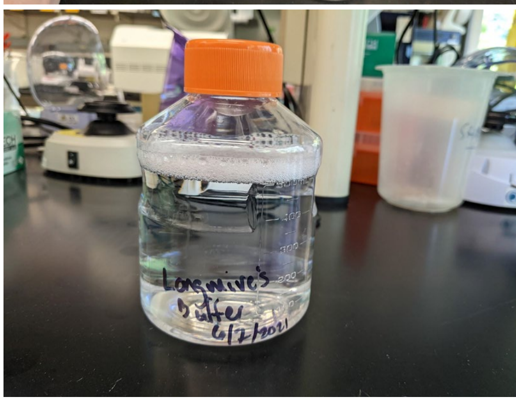




6. Cap the lower storage bottle with the provided cap, and label and date the Longmire's solution (pictures below).







Setting up the filtration system and preparing other materials

Explanation: This step involves setting up the vacuum pump, manifold, and tubing in preparation for filtering water samples. It takes less than 5 minutes to set up the filtration system and can be set up at any point during the expedition. It is recommended that the setup is put together during mobilization prior to the expedition to ensure that all of the parts are accounted for and that the system is working properly (e.g. turning on the vacuum pump). The 20 L vacuum bottle acts as the vacuum, preventing water from entering the pump. Water is poured into the cup of the 0.45 µm filter cartridge unit cup, which gets attached to the top of the adapter of the vacuum manifold. The vacuum pulls the water through the filter and subsequently into the vacuum manifold. Water exits through the Tygon tubing on the right hand side of the vacuum manifold into the 20 L vacuum bottle. The vacuum pump is then pulling air and creating a vacuum using the 20 L vacuum bottle in order to pull water through the 0.45 µm filter. Additionally, this step also involves preparing the WhirlPak bags and filling up the 5 mL Eppendorf tubes with Longmire's buffer.

Explanation: These are the supplies needed to filter the water and store the filters until they can be processed.

Supply	Quantity	Consumable
Vacuum pump	1	No
Vacuum manifold	1	No
20 L vacuum bottle	1	No
Vacuum bottle lid, 2 port, ⅜”	1	No
Tygon vacuum rated tubing, ⅜ × 9⁄16"	1	No
0.45 µm filter cartridge units	Enough to cover number of samples + 1 blank per CTD cast	Yes
69 oz. WhirlPaks	Enough to cover number of samples + 1 blank per CTD cast	Yes
Parafilm	Enough to cover number of samples + 1 blank per CTD cast	Yes
Longmire’s buffer solution	5 mL needed per sample	Yes
5 mL centrifuge vials and/or Eppendorf tubes	Enough to cover number of samples + 1 blank per CTD cast	Yes
Transfer pipettes	1	Yes
Vial rack	1	No
Vial boxes	1	No
Corks or stoppers	3	No
Adapters (typically come with the filter cartridge units and attach the filter to the stopper)	3	No
Forceps	2	No
DNA Away	1 bottle = 250 mL (1 - 2 bottles per expedition)	Yes

Labels	1 per vial (Enough to cover number of samples + 1 blank per day of sampling)	Yes
Gloves	1 pair per day/per person	Yes
Pitchers	2	No
Sharpies	1 - 2	Yes
Paper towels	1	Yes

A) **Vacuum pump**
B) **Vacuum manifold**
C) **20 L vacuum bottle**
D) **Vacuum bottle lid, 2 port, ⅜”**
E) **Tygon vacuum rated tubing, ⅜ × 9/16"**

1. If not cut already, cut three pieces of the Tygon vacuum rated tubing: two long pieces (approximately 1 - 2 meters in length) and one short piece (approximately 1 foot in

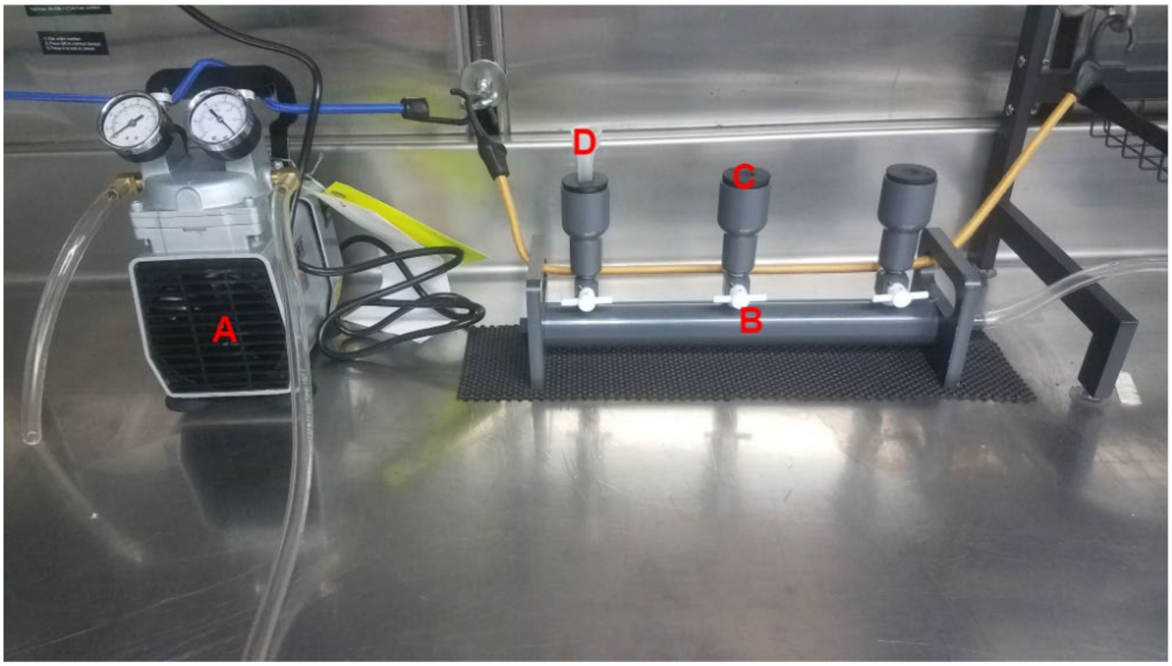
length). Attach one long piece to the right hand side port on the vacuum pump (A) to one of the vacuum bottle lid (D) ports, which sits on the floor.

2. Attach the other long piece to the right hand side of the vacuum manifold (B) and to the other port on the vacuum bottle lid (D).

3. Attach the short piece to the port on the left hand side of the vacuum pump (A).

4. Inside the vacuum pump lid, on the underside of the port leading to the manifold, attach a short (10-15 cm) section of tygon tubing. This prevents water spray from the manifold from being sucked into the tubing leading to the vacuum pump (picture below).

5. Secure all components with bungies.



A) Vacuum pump
B) Vacuum manifold
C) Cork or stopper
D) Adapter

5. Place three corks/stoppers (C) onto the manifold (B) and insert wedge adapters (D) into all three corks. Wedge the adapters in as far as they will go and ensure they are secure. You may have to make the openings of the corks/stoppers larger to accommodate the adapters by scraping away the opening of the stopper.

6. Fill one WhirlPak bag up with 2L of water from the tap. Create a line on the WhirlPak bag with a sharpie to mark that point. Use this WhirlPak bag to then create 2L line marks on the rest of the bags that will be used for the entire expedition. Throw out this WhirlPak when done using.

7. Put on gloves. Prepare the Eppendorf tubes to store filters. Fill each one with 5 mL of Longmire's buffer solution using a transfer pipette. Fill the number of Eppendorf tubes that will be used for the entire expedition. This step can also be done when creating the Longmire's buffer solution.

During the Expedition

Before the CTD cast

Explanation: Preparing the sampling strategy and supplies before the CTD is deployed. The following table contains estimates of CTD time and depth. **NOTE:** Below are sampling strategies that were used on EX-21-02 to collect water samples throughout the water column on deep (>200 m) and shallow (<200 m) CTD casts based on a sampling strategy used by the U.S. Geological Survey and the NWFSC. This sampling strategy is just a guide. The water column community should be consulted when determining any kind of sampling strategy using the CTD rosette. At minimum, water samples close to the seafloor and within and around the deep scattering layer should be collected.

Table 1. Estimated time of conducting a CTD rosette cast at 500 meter intervals.

Depth (meters)	Estimated Cast Time triggering 12 Niskin Bottles (hh:mm)	Estimated time dedicated to CTD cast; includes Dynamic Positioning, turning discharges inboard/onboard and resuming mapping survey lines
500	0:45	0:45
1000	1:15	1:15
1500	1:30	1:30
2000	2:00	2:00
2500	2:30	2:55
3000	3:00	3:25
3500	3:30	3:55
4000	4:00	4:25
4500	4:30	4:55
5000	5:00	5:25
5500	5:30	5:55
6000	6:00	6:25

1. Engage with the SST, Expedition Coordinator, and/or Mapping Lead on where the casts will be conducted, at what depth, and in determining the sampling strategy for the cast (i.e. when the Niskins will be fired). Base the sampling strategy off the depth of the location.

2. For deepwater CTD casts (> 200 m), generally follow this sampling strategy (d-e can be determined by the EK60/80 echograms; shows up best on 18 kHz and 38kHz):

    a. 1 Niskin bottle sample ~5 m off the bottom: Used to detect the eDNA of the benthos
    b. 1 Niskin bottle sample ~10 m off the bottom: Used to detect the waning concentration of the eDNA benthos
    c. 1 Niskin bottle sample ~20 m off the bottom: Used to detect the waning concentration of the eDNA benthos
    d. 1 Niskin bottle sample below the deep scattering layer (DSL)
    e. 1 Niskin bottle sample within the DSL
    f. 1 Niskin bottle sample above the DSL
    g. 1 sample in the chlorophyll max
    h. 1 sample at the surface
    i. Evenly space out the rest of the samples from seafloor to surface.

3. For shallow-water CTD casts (< 200 m), generally follow this sampling strategy:
    a. 1 sample near the bottom ~ 5 m off the bottom
    b. 1 sample at the surface
    c. Evenly space out the rest of the samples
    d. **NOTE:** All 12 Niskins do NOT need to be fired when conducting a shallow-water CTD cast. Processing samples may take longer due to the likely increase in suspended sediments in water samples, which is dependent on location. Deep water samples should take a couple of minutes to filter.

4. Have the Mapping Lead take a screenshot of the EK60/80 data before proceeding with the CTD cast and save it to the Screenshot folder on the public drive. The naming convention should be as follows: "eDNA_EK60_YYYYMMDD_depth".

5. Prepare supplies: An unopened 0.45 μm filter for each sample, a clean WhirlPak bag for each sample (with a 2L mark on it), a 5 mL Eppendorf tube filled with 5 mL of Longmire's Buffer solution in it for each sample (pre-filled earlier), forceps, gloves, blank labels, and DNA Away.

6. Label the WhirlPak bags with the corresponding Niskin bottle number (1 - 12). Label an extra WhirlPak as "blank" for the freshwater control sample.

7. Check that the vacuum bottle is empty. Empty the vacuum bottle regularly during the filtration process to prevent water from being sucked into the vacuum pump, which can damage the pump over time.

8. Create labels for each of the 5 mL Eppendorf tubes with tape or labels. Use the following naming convention for each tube: "EXYY##_CTD###_B##_S##". The naming scheme

is as follows:Expedition identifier, CTD rosette identifier, bottle number, and sample number. Sample number is included because more than one sample can be taken from the Niskin bottle (e.g. pulling 4 L of water from the CTD Niskin and treating it as two separate samples).

9. For tubes that will contain blank samples, use the following naming scheme for the vial: "EXYY##_CTD###_Blank." One blank sample should be run per CTD cast.

10. Create labels for each of the WhirlPaks. Use tape and/or a Sharpie to write down the Niskin bottle number on each WhirkPak to keep track. Also write down "blank" on another WhirlPak bag for the blank sample.

11. Put on gloves.

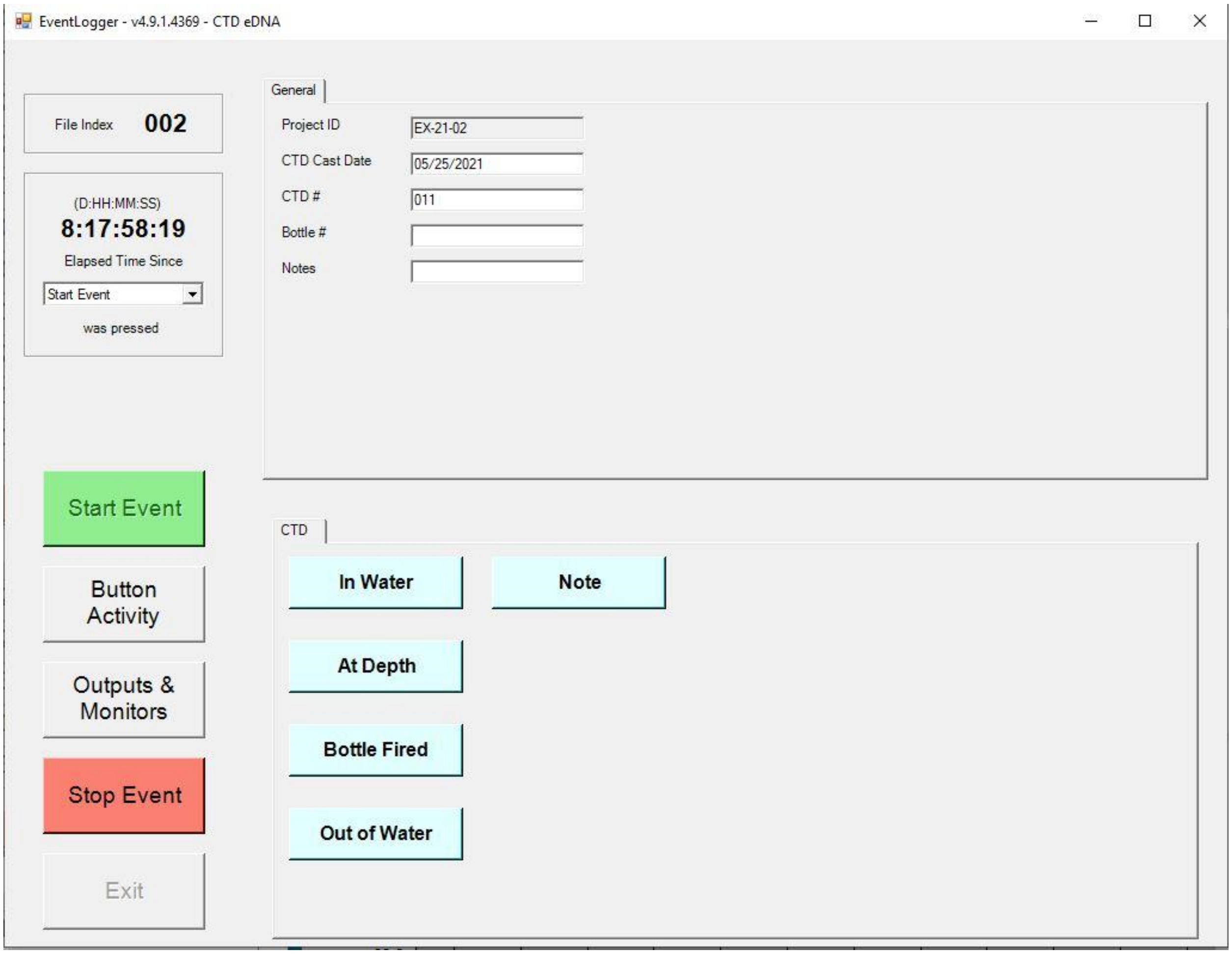
12. Clean and wipe down the benchtop and vacuum manifold with DNA Away (always wear gloves when using DNA Away).

13. Wipe forceps with DNA Away. Squirt DNA Away onto a paper towel and wipe down gloved hands as well before collecting water. Place the forceps on the paper towel that now has DNA Away on it.

During the CTD cast

Explanation: Duties during the CTD cast. Note this step involves regular communication with the SST.

1. The SST will be running the CTD cast. An extra person will be needed to log the SCS events during the cast (screenshot below).



2. Let the SST know at which depths you'd like to collect water samples. Water samples are collected on the upcast.

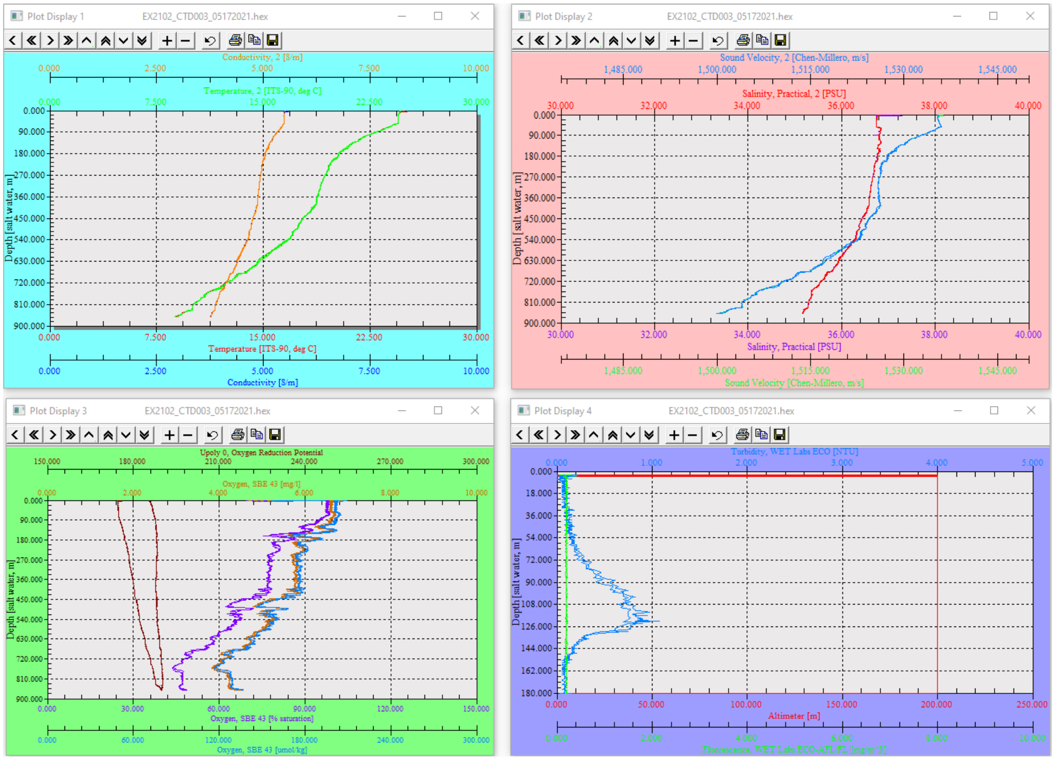
3. When the CTD rosette enters the water, on the SCS event, hit "In Water".

4. When the CTD rosette is at depth, on the SCS event, hit "At Depth".

5. When the SST fires a Niskin, simultaneously on the SCS event, hit "Bottle Fired".

6. When the CTD rosette is out of the water, on the SCS event, hit "Out of Water".

7. Once the CTD is complete, ask the SST to take a screenshot of the raw CTD data This screenshot will need to go into the CTD rosette summary form (example screenshot below).



8. Ask the SST to save the CTD data to the public drive with the following naming conventions:
    a. Level_00
        i. EXYY##_CTD###_YYYYMMDD.bl
        ii. EXYY##_CTD###_YYYYMMDD.hdr
        iii. EXYY##_CTD###_YYYYMMDD.hex
        iv. EXYY##_CTD###_YYYYMMDD.XLMCON
    b. Level_01
        i. EXYY##_CTD###_YYYYMMDD.cnv

9. As the CTD cast is being conducted, look at the quality of the data and make any notes about data spikes or bad data points.

10. Finally, ask the SST to copy and paste the events logged from the SCS event into the pertinent Google spreadsheet.

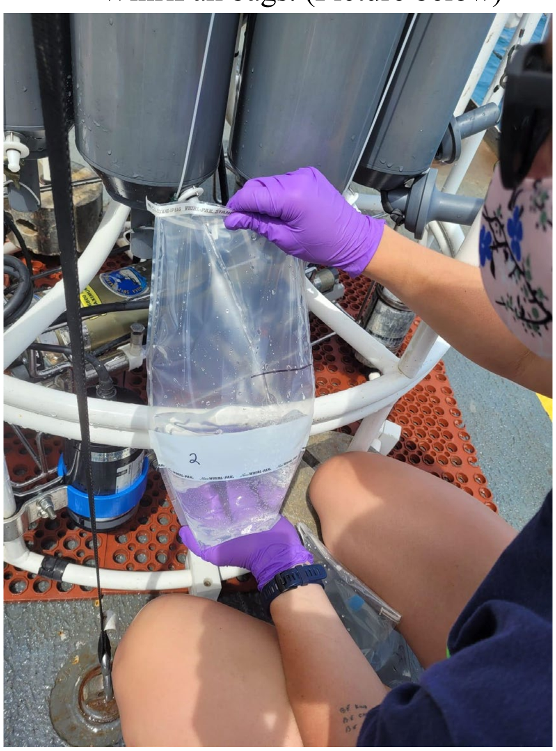
After the CTD cast

Explanation: This step involves getting the water out of the Niskin bottles. Pull the water from the Niskin bottles into the WhirlPak bags as soon as possible. Water can sit at room temperature for 1 - 2 hours in the WhirlPaks while filtration is ongoing but should be stored in the refrigerator if the filtration step is delayed longer than two hours. Gloves are required for water collection and processing.

The water samples can be stowed away and filtered at a later time if other operations are taking place. In general, the less time the water sits out before being filtered the better. The biggest concern is having the water samples directly exposed to heat and UV light. However, sitting out for an hour or so while someone works through filtering them is okay, especially if the samples are kept out of direct sunlight. If someone were going to process biological samples for a couple of hours before filtering, the samples can be retrieved from the Niskin bottles and stored in the refrigerator. They can sit in the refrigerator for up to 24 hours. The main concern is having the water sit in the Niskin bottles for an extended period of time as the Niskins could heat up and ruin the samples.

1. Put on gloves.

2. Take WhirlPaks and pitchers out to the CTD rosette. Fill 2L of water in each WhirlPak from its corresponding Niskin bottle. Ensure that the inside of the Whirlpak bags do not touch any part of the Niskin bottle. Hold WhirlPak bags in pitchers if filling up multiple WhirlPak bags. (Picture below)

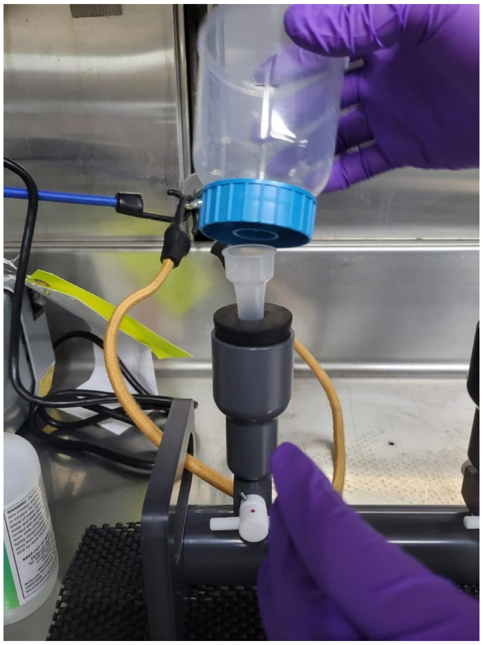


3. Close the WhirlPaks and bring them back to the wetlab and out of the sun as quickly as possible. Set up all of the WhirlPak bags in the sink or a bucket if needed. Make sure they are standing upright and that water does not spill out of the bags. (Picture below)

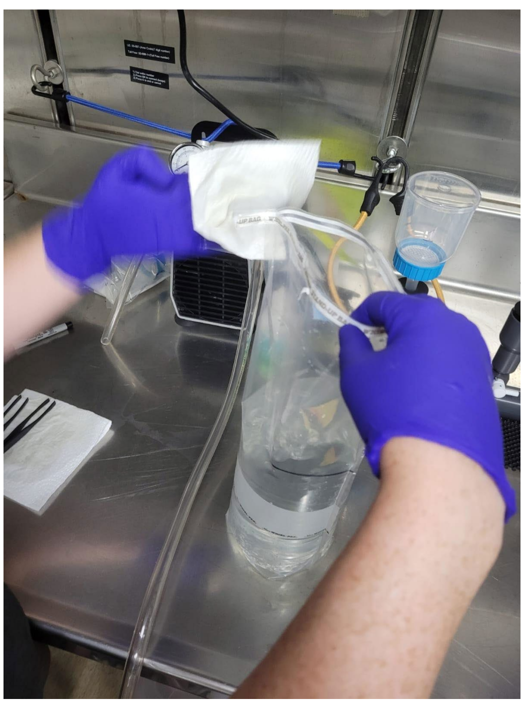
Filtering the water

Explanation: Once the water is removed from the Niskins, these next steps involve filtering and storing the water. Three water samples can be processed at the same time if there are multiple people around to assist with pouring water into the 0.45 µm filter cartridge unit. If only one person is filtering, filter one sample at a time. **NOTE:** There are two types of filters used to filter water samples. They contain the same filter pore size (0.45 µm), but are different brands.

1. Put on gloves.
2. Remove the 0.45 µm filter cartridge from the packaging and write the Niskin bottle number on it. Attach it to the adapter. The adapter is attached to the stopper and the stopper is plugged into the vacuum manifold. (Picture below)

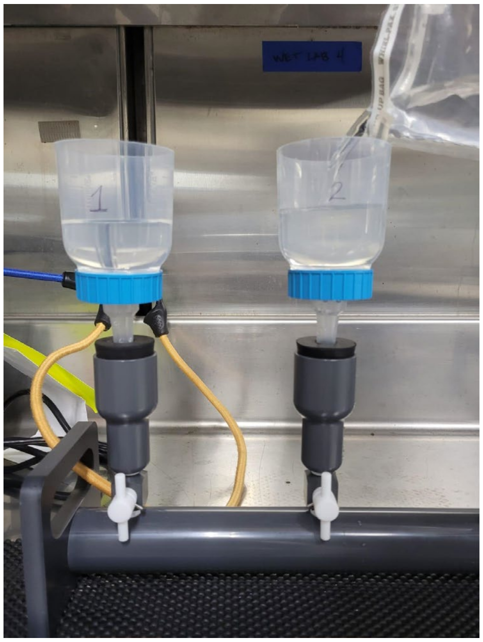


3. Wipe down the opening of the WhirlPak bag where the water will be poured out with a paper towel that has DNA Away on it (picture below). Wipe down gloved hands with DNA Away. Forceps should have already been wiped down with DNA Away and they should be **DRY**.

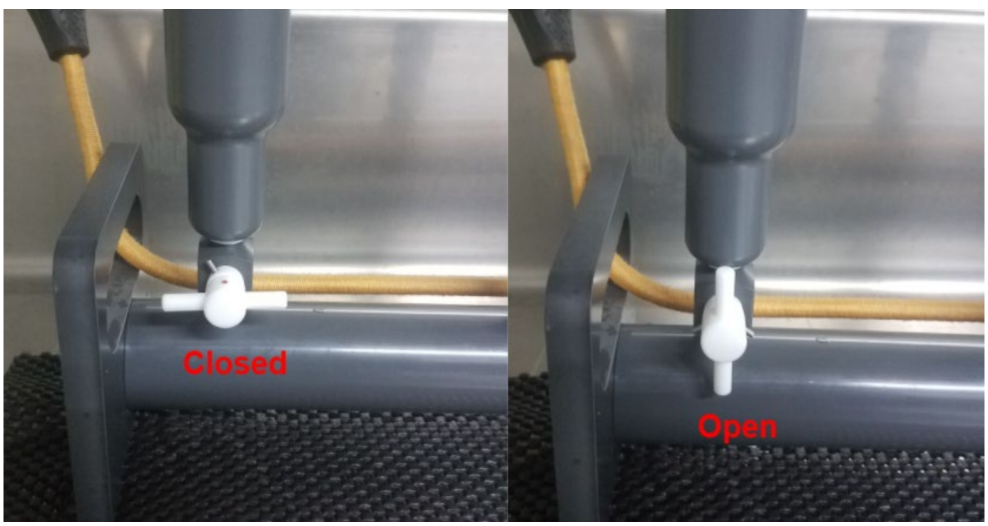


4. Take the lid off the top of the filter cartridge and pour water into the top. (Picture below)

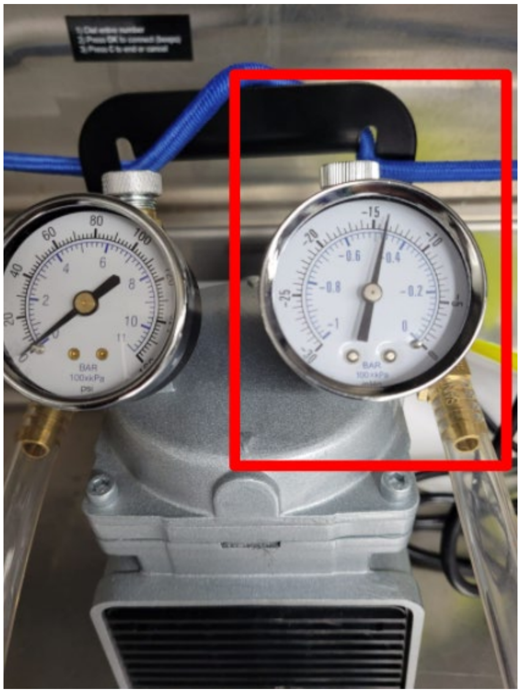
a. **NOTE:** The picture below shows the valve in the open position already. When first pouring water into the filter cartridge, make sure the valve is closed first.



5. Start the vacuum pump and open the valve on the manifold. (Picture below, left is closed and right is open)

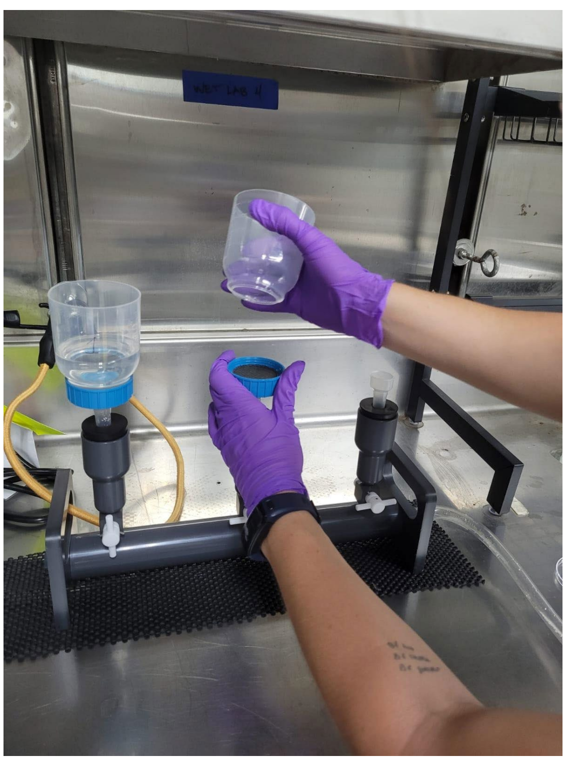


6. As the water is pulled through, gradually add more water into the top of the filter cartridge. Don't let the filter run dry. Ensure that the vacuum pump does not go above -15 inHg (inches of Mercury). Adjust the knob above the display to adjust the inHg (picture below).



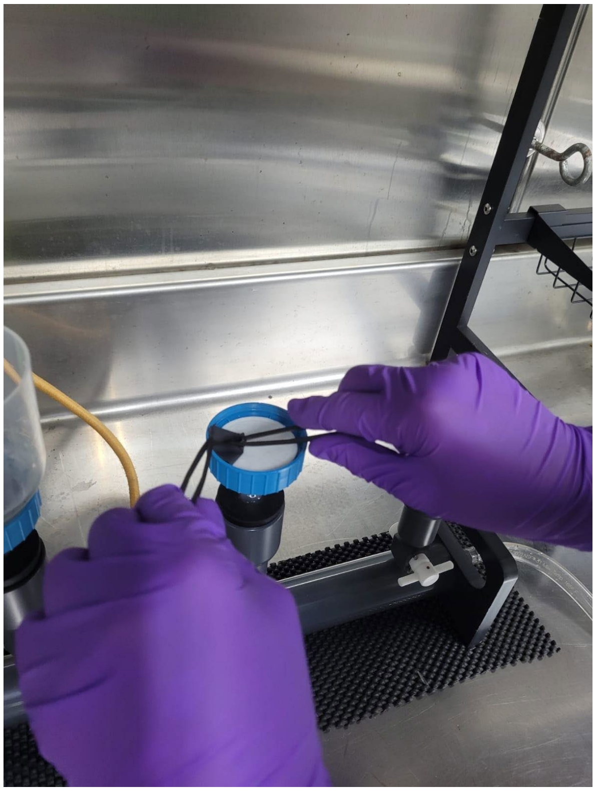
7. Once you have filtered all of the water in the WhirlPak, close the valve and shut off the vacuum.

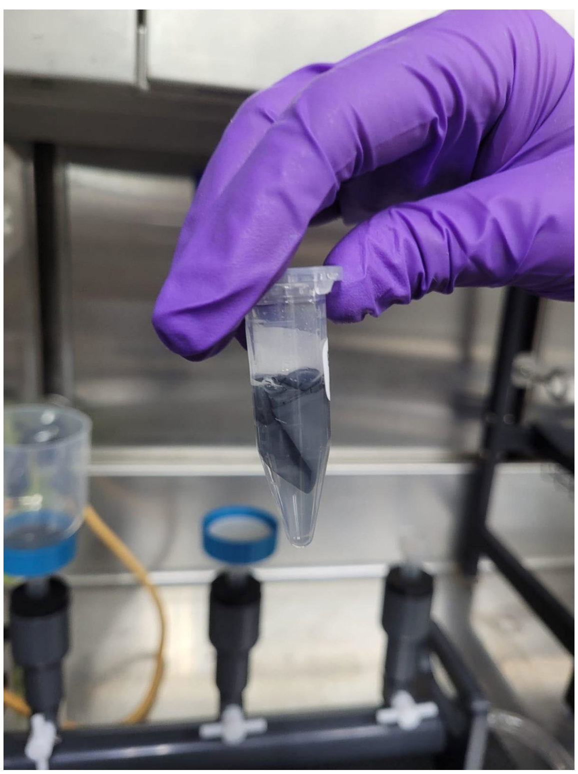
8. Gently snap off the filter cup from the filter lid — do NOT twist (picture below).



9. Clean your gloves with DNA Away.

10. Using the cleaned forceps, carefully fold or roll the filter to fit into the labeled 5 mL Eppendorf tube. Grasp the filter with the forceps and place it in the tube, making sure it is fully submerged in the buffer. (Series of pictures below)
    a. **NOTE:** There is a white pad underneath the actual filter that should NOT be collected.





11. Close the lid on the vial and wrap with parafilm to seal. Place in the box and store in a dark place (keep vials out of the light as much as possible).

12. **STOP:** Check the vacuum bottle. If it is halfway full of water, empty it now before proceeding to the next sample.

13. Wipe down gloves, forceps, and the outside of the next WhirlPak with DNA Away.

14. Repeat steps 1-11 for each additional sample.

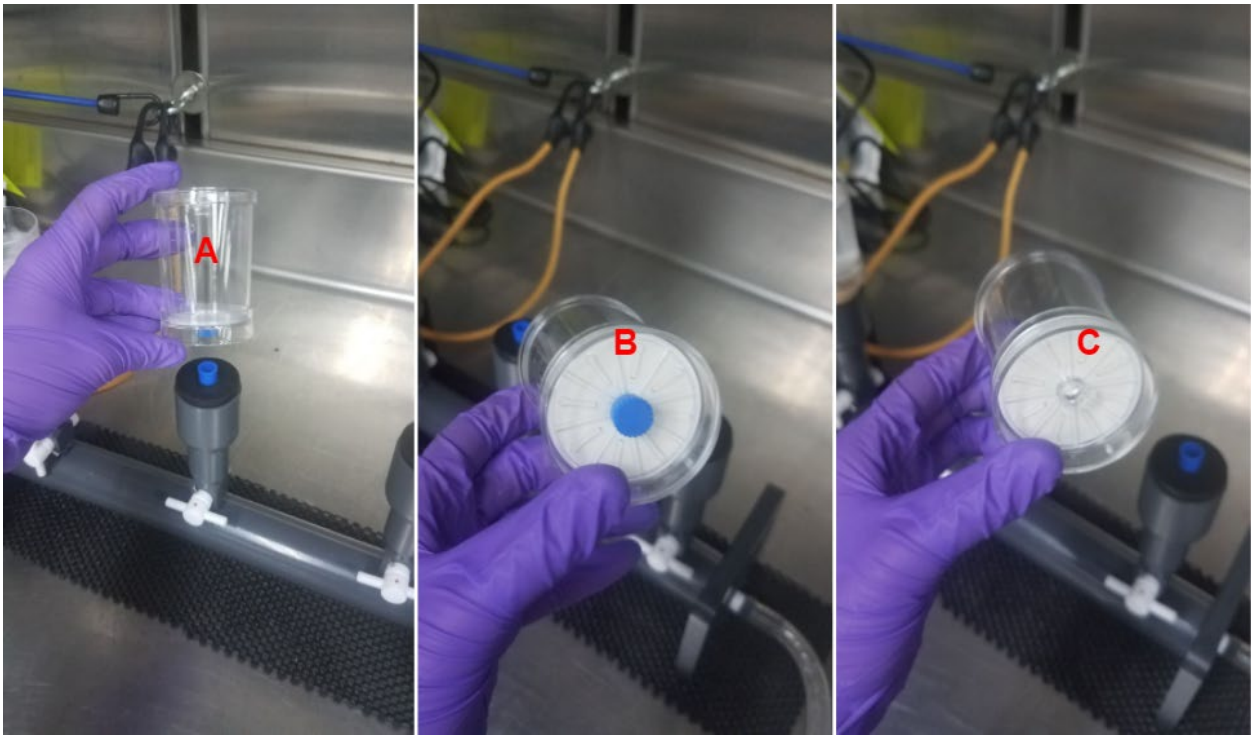
15. After running all of the samples, fill up a WhirlPak bag with 2L of freshwater from the sink and repeat steps 1-10 with the freshwater water. This is the control, or "blank", sample for the group. Run one "blank" sample per CTD cast.

16. After all samples are completed, pour freshwater into the tops of the adapters to clean out the manifold while the vacuum pump is running.

17. Unplug the Tygon tubes from the top of the vacuum bottle vacuum lid and empty the vacuum bottle into the sink. Keep the vacuum pump running for 5-10 minutes to pump some air through the system to help keep it dry.

18. Turn off the vacuum and clean up the workspace.

NOTE: Pictured above are 0.45 µm filter cartridges from Fisher Scientific. These filters were used up on the ship. Below are pictures of 0.45 µm filter cartridges from Sterlitech. The brand of filter cartridges may be different, but the procedures listed above for filtering water are exactly the same.



A) Sterlitech filter cartridges
B) Bottom of the cartridge filter, with a blue cap covering the bottom port of the cartridge
C) Remove the blue cap and stick onto the adapter to filter the water

Filling out the CTD Rosette Summary Form

Explanation: Data management priorities after the CTD rosette cast and filtering the water samples.

1. After filtering the water, fill out the CTD Rosette Summary Form. The form documents information about the cast including coordinates, times, maps, plots, etc. Work with the SST to complete the form if necessary (e.g. documenting any problems with the sensors). Most of the data in the form can be found in the SCS metadata Google spreadsheet that the SST updated after the completion of the cast.
    a. A CTD Rosette Summary Form should be completed for every CTD cast conducted.

2. Work with the Expedition Coordinator and/or Mapping Lead to ensure that the CTD data and summary forms become a part of the expeditoin data package.

3. Final products checklist:
    - Ensure the CTD data made it to the public drive and has the correct naming convention
        - Ensure there is Level 00 and Level 01 data
    - CTD Rosette Summary form is complete and contains all pertinent information
        - Ensure there is a screenshot of the EK data in the form
        - Ensure there is a screenshot of the CTD data in the form
    - CTD Rosette metadata is recorded in its respective Google spreadsheet
        - EXYY##_CTD
    - SCS metadata is recorded in its respective Google spreadsheet
        - EXYY##_CTDrosette_WaterSample_Metadata

Data Delivery and Reporting

1. In the corresponding expedition report (or mapping report if a dedicated mapping expedition) the following tables with the following columns will be reported:
    - **EX-YY-## CTD summary table** with fields: CTD name, CTD number, data (UTC), deployment latitude (decimal degrees), deployment longitude (decimal degrees), recovery time (UTC), recovery latitude (decimal degrees), recovery longitude (decimal degrees), max depth (meters)

Example:

CTD Name	CTD Number	Date (UTC)	Deployment Time (UTC)	Deployment Latitude (decimal degrees)	Deployment Longitude (decimal degrees)	Recovery Time (UTC)	Recovery Latitude (decimal degrees)	Recovery Longitude (decimal degrees)	Max Depth (meter)
EX2102_CTD001_20210515	CTD001	15May2021	10:26:49	28.38146	-80.51023	10:32:57	28.38146	-80.5102	7.729

- **EX-YY-## Inventory of water samples** collected with fields*: CTD number, sample name, data, time (UTC), depth (meters), temperature (°C), salinity (PSU), dissolved oxygen (mg/l), oxygen reduction potential (mV), density (kg/m^3), fluorescence (mg/m^3), turbidity (NTU)
    - *Environmental sensors are subject to change based on sensor operability and equipment used

Example:

CTD#	Sample Name	Date	Time (UTC)	Depth (m)	Temperature (°C)	Salinity (PSU)	Dissolved Oxygen [mg/l]	Oxygen Reduction Potential (mV)	Density (kg/m³)	Fluorescence (mg/m³)	Turbidity (NTU)
CTD001	EX2102_CTD001_B01_S01	15May2021	10:30:00	7.452	24.9792	35.626	6.4381	229.6703297	23.853	NA	NA

2. The following data and tables must be delivered to NOAA's National Centers for Environmental Information (NCEI) via the OER Internal Products and Services Form (you can share a link to the Google Drive cruise folder) within three weeks of expedition completion.
   - CTD and sensor calibration files, example here.
     - These must be submitted with every data set
     - The calibration files can be requested from the Senior Survey Technician
   - Level_00, example here.
     - Files: *.xmlcon, *.hx, *.hdr, *.bl
   - Level_01, example here.
     - Files: *.csv
   - The tables above, EX-YY-## inventory of water samples and EX-YY-## CTD summary table. These can be in Google Sheet format.
   - Completed CTD Summary Forms, example here.

3. The eDNA sequencing will take 1-3 months post-expedition to complete. Once it is completed, the analyst will send a species/biological inventory (*.csv). This sheet is then sent on to NCEI for archival to be cross referenced with the rest of the expedition data.

4. The raw sequencing data is submitted to the National Center for Biotechnology Information (NCBI) Sequence Read Archive where they will be publicly available to downstream users. Once a URL/DOI is available, this can be shared with NCEI for cross-linking with the rest of the expedition data.